Examining anaerobic microbial communities that direct the fate of terrestrial carbon in lake sediments

by

Kurt M. Yakimovich

A thesis submitted in partial fulfillment
of the requirements for the degree of
Masters of Science (MSc) in Biology

The Faculty of Graduate Studies
Laurentian University
Sudbury, Ontario, Canada

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian Université/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	Examining anaerobic microbial communities that direct the fate of terrestrial carbon in lake sediments
Name of Candidate Nom du candidat	Yakimovich, Kurt
Degree Diplôme	Master of Science
Department/Program Département/Programme	Biology
Date of Defence Date de la soutenance	June 05, 2017

APPROVED/APPROUVÉ

Thesis Examiners/Examinateurs de thèse:

Dr. Nathan Basiliko
(Co-Supervisor/Co-directeur de thèse)

Dr. Nadia Mykytczuk
(Co-Supervisor/Co-directrice de thèse)

Dr. Andrew Tanentzap
(Committee member/Membre du comité)

Dr. Carl Mitchell
(External Examiner/Examinateur externe)

Approved for the Faculty of Graduate Studies
Approuvé pour la Faculté des études supérieures
Dr. David Lesbarrères
Monsieur David Lesbarrères
Dean, Faculty of Graduate Studies
Doyen, Faculté des études supérieures

ACCESSIBILITY CLAUSE AND PERMISSION TO USE

Abstract:

Freshwater lake sediments play key roles in the cycling of carbon. This includes hosting microbial communities responsible for mineralizing large amounts of carbon into greenhouse gases—namely CO_2 and CH_4. Yet despite the important roles of sediment communities, their ecology and community structure linkages to biogeochemical cycling are not well known, and little data exists exploring how sediment microbial communities respond to different organic matter inputs. Here I start by reviewing previous literature on lake sediment microbial communities and the physicochemical factors affecting their composition and diversity. Next I report on data from two experiments, first an in-vitro lab study and then an in-situ field study, in which lake sediments were amended with different plant litters that could result from land use change and or succession in catchments. Microbial communities were examined with next generation amplicon sequencing. These data were linked to rates of CO_2 and CH_4 flux and dissolved organic matter (DOM) components present in pore water were examined as potential controls on community structure and function. I observed *in-vitro* that methanogen community composition and activity were affected by OM type, with macrophyte derived C enhancing microbial activity, whereas high concentrations of polyphenolic compounds from terrestrial tree litters inhibited methanogen activity. The polyphenols had an environmental filtering effect, selecting for different bacteria, fungi and methanogen communities. The *in-situ* experiments involved installing mesocosms with artificial sediments with variable amounts of deciduous and coniferous tree leaf litter. In these mesocosms we observed a link between methanogen community composition and decomposition rates, as measured with bulk CO_2 and CH_4 production and DOM humification. Decomposition rates were influenced by lake physicochemical factors, particularly the degree of photoexposure. With increased

decomposition, specialist taxa of methanogens could thrive that conferred higher rates of methanogenesis. The work presented here demonstrates the adaptability of methanogen lake sediment communities as terminal decomposers under changing terrestrial OM subsidies.

Keywords:

Sediment, microbiome, bacteria, fungi, methanogens, freshwater, microbiology, leaf litter, decomposition, lake sediment, carbon, methane.

Acknowledgments:

I'd like to acknowledge both of my supervisors Drs. Nadia Mykytczuk and Nathan Basiliko who have treated me well and have provided an excellent educational opportunity for me to broaden my research skills. I also want to thank my fellow lab members and all the other staff and students at the Living with Lakes Centre for helping with various things over the years, and provided an atmosphere that often felt like a home away from home.

I also want to acknowledge my collaborators from over the pond at Cambridge University, Dr. Andrew Tanentzap and his research group that includes Dr. Erik Szkokan-Emilson, Chloe Orland, Cyndy Desjardins, Beth Smith and others whom I have worked with during my time at the Lake Centre. Lastly, I would like to thank the Laurentian Biology department and Graduate studies who helped facilitate my degree.

Table of Contents

List of Figures:

List of Tables:

List of Appendices:

General introduction:

Lakes form an important link in the global carbon (C) cycle by acting as a conduit for terrestrially derived C from plants, which either gets buried in sediments or mineralized and returned to the atmosphere. Although this represents a natural cycle, human induced global climate and land use change are altering the C cycling across integrated terrestrial and aquatic systems. This is important as terrestrial C can be mineralized both to CO_2 and CH_4, with the latter being ~25 times more potent as a greenhouse gas (Forster *et al.*, 2007). This means that there is not an even exchange of radiative forcing if plants fix CO_2 but tissues are even partially decomposed to CH_4. With the recent realizations that freshwater systems receive 2.9 Pg of C annually, their functioning is becoming recognized as an increasingly important area of study (Tranvik *et al.*, 2009).

Responsible for directing the fate of C in lakes are the sediment microbial decomposers including bacteria, fungi and archaea (primarily methanogens). The highest densities of microbial biomass generally occur in the sediments, which are the primary site for methanogenesis in lakes. These microbial communities respond to changing C sources and input rates by changing in biomass and/or composition as their community adapts, however little literature exists on how littoral bacterial, fungal and methanogen sediment communities respond to different plant litter inputs. Therefore, my MSc thesis work involved two novel and complementary studies to provide insights on this topic. The scope of this thesis includes a review of relevant literature, a well constrained incubation experiment, and a field experiment, which have been written in manuscript formats. The thesis chapters are presented in manuscript format, and list co-authors on each chapter's title page.

Chapter 1 consists of a literature review on the ecology of microbes in freshwater lake sediments. The scope of this review extends beyond the topics of the other two chapters, but still provides adequate background information on the overall topic of this thesis. The focus of the review was to gather all relevant literature into one document and synthesize what is known about lake sediment communities with an emphasis on guilds that play key roles in C and GHG cycling. This includes evaluating different C sources as well as different lake physicochemical factors that affect microbial community composition and functioning.

Chapter 2 is a primary research chapter based on lab incubation experiments used to examine how different plant litters affect microbial community composition. Lake sediments were amended with one of three plant litter treatments: a deciduous mixture, a coniferous mixture or cattails (*Typha latifolia*). These different litters represent differing sources of C for sediment microbial communities and the amounts of these litters entering lakes can change with human land use practices as well as plant community responses to climate change. I used amplicon sequencing and performed qPCR on the incubated samples to assess changes in the bacterial, fungal and methanogen communities with the different plant litter additions. The primary research objective of this chapter was to ascertain basic information on the shifts in microbial community composition in response to different plant litter types and any associated physicochemical factors, and in turn CH_4 and CO_2 production rates

Chapter 3 focusses on field experiments conducted in three lakes in Sudbury ON, Laurentian Lake, Ramsey Lake and Swan Lake. 30 mesocosms were placed in each lake containing artificially constructed sediments representing concentration gradients of both deciduous and coniferous leaf litters. The sediments from these mesocosms were sampled once a month from August to October 2015. The methanogen communities were analyzed using

amplicon sequencing, and both the temporal dynamics across all three lakes as well as the effects of the different plant litter additions was examined. The objective of this chapter was to evaluate the results from the incubations in chapter 2 *in-situ*, using a comparison of 3 different lakes to help tease out physicochemical factors important to shaping the microbial communities and directing the fate of C to CH_4 in lake sediments.

References:

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey DW, *et al.* (2007). 2007: Changes in Atmospheric Constituents and in Radiative Forcing. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, et al. (eds). Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA. https://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter2.pdf.

Tranvik LJ, Downing J a., Cotner JB, Loiselle S a., Striegl RG, Ballatore TJ, *et al.* (2009). Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol Oceanogr* **54**: 2298–2314.

Chapter 1: Lakes as globally important microbial habitats: an ecological overview of lake sediment microbiomes

Kurt M. Yakimovich[1,2], Chloé Orland[3], Andrew J. Tanentzap[3], Nathan Basiliko[1,2], Nadia C.S. Mykytczuk[1,2,4]

[1] Vale Living with Lakes Centre, Laurentian University, Sudbury ON, Canada
[2] Department of Biology, Laurentian University, Sudbury ON, Canada
[3] Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom
[4] School of the Environment, Laurentian University, Sudbury ON, Canada

Abstract:

Freshwater lakes and other inland water systems play an important role in regulating global carbon (C) cycles and therefore global climate; and vital to this cycling are the sediment microbial communities. C cycling by planktonic communities has been extensively studied, however it is only more recently that there has been a surge of interest in C and methane (CH4) cycling by sediment microorganisms. Lakes have different zones based on depth and proximity to shore, each with different physicochemical characteristics, and sediment microbiomes are neither homogenous horizontally nor vertically across a lake. Thus, lake microbiomes differ in composition, abundance and function in regards to directing the fate of carbon from littoral to profundal zones. Due to the accumulation of leaves, detritus and animal remains in the near-shore environment, catchment areas and riparian zones of lakes are key to the initial decomposition of terrestrial organic matter (TOM), and to colonization by bacteria and fungi. The TOM deposited on the lake bottom is an important source of carbon for sediment microbial communities, fueling methanogenesis and transferring carbon higher into aquatic food webs. In some cases, littoral sediments can be a large source of CH_4 efflux to the atmosphere, making them important for global carbon budgeting. Methanogenic communities in the top ~0-5cm of sediment are largely responsible for the total methanogenesis in lake sediments and have relatively stable biomass temporally and horizontally. The bacteria in the top layer are far more dynamic and respond to changes in environmental factors and resources that vary primarily at seasonal scales, particularly OM and other nutrient inputs. Vertical profiles of lake sediments reveal distinct layers of the sediment separated by distinct microbial communities. In the case of archaea, the profile shifts to largely unknown taxa, and in general biomass decreases. However, in some lakes, archaea remain near constant from the top of the sediment to as far as 38 cm deep.

The different zones explored here represent unique microbial habitats in lake sediments, and this is reflected by varying community structure and abundance, with consequences on carbon cycling.

Introduction:

The microbial inhabitants of a lake are largely responsible for directing the fate of organic carbon entering the system. Their community structure and functional abilities vary across different zones of a lake. This heterogeneity must be accounted for when doing research, as the different zones represent different microbial habitats governed by differing physicochemcial factors that change the cycling of carbon. These finer scale microbial dynamics of freshwater lakes and water bodies are important to understand as they are directly connected to global carbon cycling. This is through the movement of carbon between the atmosphere, terrestrial systems and freshwater systems. Lakes have a disproportionate role in global carbon cycling, considering their global area [1]. In particular, freshwater systems play a huge role in the cycling of terrestrial carbon, suggesting the current description of this cycle should be revised to include terrestrial-aquatic linkages[1]. Underscoring this is the fact that nearly twice the amount of terrestrial C enters freshwater systems than the ocean, and about half of that terrestrial carbon input is returned to the atmosphere [1,2]. As the terrestrial organic matter (TOM) along with aquatically derived organic matter (referred to hereafter as Lake derived Organic Matter—LOM) is decomposed, much of it gets mineralized through microbial respiration, which in most lakes exceeds carbon fixation rates by primary producers [3–6]. It is estimated that the combined emissions of CO_2 and CH_4 from freshwater sources equal 79% of the estimated land greenhouse

gas (GHG) sink. Freshwater microbes are therefore active and crucial participants in global carbon cycling and as climate regulators [6-7].

With Earth's 304 million freshwater lakes, it is important to understand how lake sediment's microbiome (LSM) is able to direct the fate of carbon [8]. The term microbiome is used here to encapsulate both the microbial fauna and abiotic conditions (i.e. temperature, nutrient levels, O_2 concentrations, pH etc.) that define lake sediments as a unique habitat. From an ecosystem level view, an LSM is responsible for the decomposition of organic matter and either mineralizes it, assimilates it, or partially decomposing it contributing to the production of complex recalcitrant organic molecules such as humic or fulvic acids. This results in two fates for carbon: (1) it escapes to the atmosphere after being mineralized — in the form of CO_2 and CH_4 — through respiration by microbes or by organisms from higher trophic levels, (such as zooplankton, invertebrates and fish) (2) or it gets stored in lake sediments via flocculation or sedimentation processes. Both fates have implications for the lake ecosystem, and must be considered when examining microbial roles in local ecosystem functioning. With the variation of the quality and quantity of OM across different zones of a lake, different microbial habitats are established (Fig. 1). To understand microbial processing of C, we must also examine the carbon sources of lake sediments.

The focus of this review will inform environmental stewardship and our understanding of the impacts of climate change. Warming temperatures are likely to increase microbial activity, which would lead to greater mineralization and evasion of carbon, rather than it being sequestered in lake sediments [9,10]. By altering sediment microbial and macrofaunal communities, warming temperatures could also dramatically modify aquatic ecosystem functioning [11]. With an estimated 820 Pg of C stored in lake sediments, we need to understand the potential for these

stores to be mobilized via microbial activity [1,12]. It is now urgent to uncover the links between the LSM and local scale ecosystem functioning, and their integration into global carbon cycling. This knowledge will allow for responsible management of our world's lakes. The purpose of this review is to examine what we know of LSM, and their role of organic carbon (OC) processing and directing of its fate.

Fueling a lake microbiome:

Primary producers from bother terrestrial and aquatics systems contribute to fueling a lake microbiome through (TOM and LOM) (Fig.1). TOM accumulates in catchment and riparian areas where is washed into lakes in various size fractions (i.e. as dissolved OM (DOM), particulate OM (POM) or even larger pieces of plant material). DOM is the most dominant type of OM in lake waters, whereas POM has the higher rates of sedimentation and dominates in lake sediments[1]. Studies have found that LOM sources of OM are preferentially degraded, which can lead to a large majority of standing stock of dissolved organic carbon (DOC) in lakes being from terrestrial sources [13]. So, while a majority of lake pelagic OC is of autochthonous origin, less labile forms of OM become buried in the sediments, and these are often of terrestrial origin [14,15]. This results in lake sediments becoming a more specialized environment for heterotrophic microbes, relying primarily on sedimented POM and humified substances as an OC source to fuel metabolisms.

Research has suggested that characterizing C transport (and related transformations) between land and water is essential for understanding both terrestrial and aquatic food webs and ecosystem functioning via nutrient and OM speciation, utilization and loss[16]. Input of OM from terrestrial sources is vital for the functioning of a lake in terms of resources available and habitat

development; and is influenced by the characteristics of catchment and riparian areas. For some lakes, litter fall within 10m of the shoreline can become a primary energy source for littoral food webs [17], however this is not always the case and varies between lakes [18]. It is therefore necessary to review the existing literature on microbial responses to different OM sources to obtain the complete depiction of an LSM over space and time.

Local scale functioning (Mineralization of Carbon):

An important group of microbes implicated in the mineralization of carbon are the methane-producing (via methanogenesis) anaerobes, the methanogens. Methanogens are gaining more attention with numerous research and review articles highlighting the role CH_4 plays in the cycling of carbon in lakes and on the larger landscapes, with methanogens being responsible for 10-50% of total carbon mineralization in anaerobic systems [1,7,9,19–22]. However, CH_4 is severely understudied relative to CO_2 in lakes and we lack a comprehensive understanding of the roles methanogens play in a LSM, and ultimately how they affect the fate of carbon in lakes. It is estimated that freshwater lakes are responsible for 6-16% of non-anthropogenic CH_4 emissions, despite only covering ~0.9% of the Earth's surface [8,22]. Furthermore, CH_4 is about 25 times more potent as a greenhouse gas then CO_2, so understanding the ecology of methanogens in lake sediments becomes key in understanding the flow of C through lake ecosystems.

Included in the understanding of the flow of C through lakes is understanding Methanotrophy (oxidation of CH_4). More research is now going into understanding CH_4 cycling in lake waters and sediments. The discovery of anaerobic oxidation of methane (AOM) has revealed sediments as important sinks for internally produced CH_4, coupling them to lake ecosystem processes (i.e. fueling food webs) and global carbon cycles and climate [19,23–26]. AOM

has been linked to both Bacteria and Archaea, with three different pathways that utilize different electron acceptors, sulfate, nitrite/nitrate or metal ions (Fe^{3+} and Mn^{4+}) [24]. The set of microbes involved are specialists that are adapted to utilizing low energy pathways to survive, and make use of substrates available in the environment.

Methanogens are the most studied Archaeal functional group but the diversity of bacteria found in sediments is far greater. Bacteria have been implicated in the cycling of nutrients in lakes, such as S, N and P. Bacterioplankton have also been linked to pelagic food webs in lakes, but there is little research providing evidence of the link between LSM and pelagic food webs [16]. With the advent of next generation sequencing techniques and better bioinformatical tools for analyzing sequencing data, lake sediment bacterial communities are becoming the subject of more in-depth study [9,27–30]. In a mesocosm experiment, Harrault *et al*. [30] found that sediment that contained more labile C sources had a greater effect on pelagic biology. This was the consequence of a greater release of phosphorus, OM and other nutrients to the water column, which lead to a higher seston biomass [30]. Additional studies have used stable carbon isotope analysis to show links between methane-derived carbon and higher trophic levels [31–35].

Littoral zones and shallow lakes:

Earth's lake are predominately small and shallow (<1km^2), and are an interface between terrestrial and aquatic systems, often acting as a conduit for the influx of TOM into lake ecosystems [8]. Sediments in the littoral zone can therefore be subject to higher quality (less decomposed) TOM influx from catchment areas (zone1 in Fig. 1). There may also be a greater flux of algal biomass mixing into these sediments via wave turbation, as well as more benthic algae and macrophytes. While the amounts of any of these OM sources depends on a variety of

abiotic factors influencing the lake characteristics, it is important to explore the LSM in the littoral zone to understand how these labile OM sources are processed by microbes and to follow the fate of the carbon. Data shows increased mineralization rates in littoral zones compared to profundal zones. With a high ratio of sediment:water depth, littoral zones typically have large amounts of sediments, with high anaerobic microbial respiration [36].

CH_4 cycling by methanogens, methanotrophs and their syntrophic partners is key in the microbial processing in the littoral zone. Escape of CH_4 to the atmosphere occurs via ebullition, plants, diffusion, seasonal advection and microbubbles [26,37]. It was previously thought that much of the CH_4 produced in anoxic sediments was oxidized by methanotrophs. However, newer evidence suggests that up to 100% of methane produced in littoral zones can escape to the atmosphere – this idea has been termed the "epilimnetic shortcut" [19,38]. It occurs following turbulence in the sediment due to natural or human induced waves, which reduce CH_4's residence time in the overlying oxygenated waters; thus prevents the oxidation of CH_4 and changes the fate of carbon. The evasion of carbon to the atmosphere is a clear indicator of how microbial metabolism in sediments is directly linked to global carbon budgets. This makes littoral sediments and shallow lakes an important microbial habitat to study, with far reaching ecological feedbacks and global relevance.

Fungi in lakes:

The catchment and littoral areas represent transition zones. In this transition from forest to lake ecosystem, carbon isn't the only entity that flows into the aquatic system: microbes, including taxa associated with terrestrial ecosystems, make their way into lakes [43]. Among these transient taxa are some Fungi species. Despite having potentially important roles as decomposers

in lake sediments, little is known about their function in these systems [40]. Fungi are known to be important decomposers of leaf litter, and other TOM with decomposition process beginning with the colonization of filamentous fungi [40]. Leaf- and soil-associated fungi stay with the TOM as it moves through catchment areas, and is believed to play a role in aquatic systems as well [40,41]. There is a shift in the primary constituents of the fungal community: from filamentous to unicellular fungi—chytrids and yeast—in the pelagic zones of lakes. However, the metabolic functions of the deep sediment fungi are unknown [40]. This shift between morphotypes is hypothesized to depend on the micro-habitats created in the lake by the quantity of fine POM and coarse POM [40]. More work has been done in deep marine sediments, where fungi have been found to adapt to higher pressure and to the extremes of living in deep sea sediments [42]. There is a need to further study the activity and role of the fungi found in these OM-limited environments. Next generation sequencing offers an avenue into better exploring fungi community structure, key organisms in our understanding of lake sediments [43].

Prokaryotic community distribution:

Many studies looking at LSM don't differentiate between littoral and profundal zones; however, as previously discussed, there are differences in carbon cycling within these two zones (i.e. proximity to TOM influxes, CH_4 efflux due to wave action); it remains unclear how these differences are related to the LSM. Research has largely focused on identifying the effects that physicochemical factors have on the LSM, but the factors that define the microbial habitat and dominant groups of microbes are still undefined. For example, a survey of 13 lakes on the Yunnan Plateau in China only analyzed cores from the center of the lakes and reported a dominance of *Proteobacter*, and large constituency of *Chloroflexi* [44]. However, this wasn't

consistent across all lakes, and the variation in community wasn't directly explained by their measured environmental variables. The authors did note that nitrate was the largest driving factor of bacterial community distribution, which they contrasted to Lake Dongping, China, where total phosphorous was the driving factor [44,45]. Also linked to LSM composition is OM source [46–49], and linking OM source to the microbial species involved in decomposition could therefore be an efficient method of defining a microbial habitat.

Although the factors defining microbial habitats aren't clear, patterns exist in the literature in microbial distributions. Studies of whole lake bacterial distribution in the shallow Lake Võrtsjärv showed that the top 5-6 cm of sediment along a 13 km transect was homogenous [39]. This result was hypothesized to be a result of wind-induced resuspension, showing that climate and topographic conditions at least partially define physical characteristic of microbial distribution patterns [39]. Another study on that same lake showed a seasonal succession of bacteria in the top 1 cm of the sediment, with productivity of sediment bacteria decreasing by 40% under the ice [50]. So, although the top layer of sediment is mixed in Lake Võrtsjärv, it shows seasonal changes in habitat conditions. The studies on this lake showed bacterial community composition and abundance responded more to other environmental and physical factors superseded proximity to shore and sources of TOM. This is in contrast with a study on Lake Erie which found differing metabolic profiles of littoral vs. profundal sediments and provided and linked these differences to DOM source and composition [48]. Additionally, data from a study on Lake Taihu demonstrate that sediment bacterial composition can respond to biotic changes such as algal blooms [49]. The results from this study agree with previous research suggesting that bacterial communities are sensitive to temporal changes that bring changes in OM sources and temperature; whereas archaeal communities have been found to be shaped by trophic level and

aren't impacted by the same shifting conditions as bacteria [44,49,51]. Bacterial communities therefore seem to shift to a metabolically favorable active community, and best utilize available resources (e.g. shifting OM sources). This suggests archaea in this system are dependent on syntrophy with bacteria to acquire metabolites, as they remain more static in their community composition—and indeed Archaea-bacteria relationships have been well explored. The bacterial communities in sediments are known to contain syntrophic partners of the methanogens, involved in the degradation of OM. Culture experiments have showed that the dominant bacterial sugar users in a lake are fermentative anaerobes that only grow in co-culture with methanogens [52,53]. These bacterial syntrophs are a part of two functional groups that have been identified as key to this: the primary and secondary fermenting bacteria [52,54]. These groups are responsible for the further degradation of polymeric molecules including nucleic acids, lipids, amino acids, polysaccharides and other monomeric biomolecules. These processes result in the availability of metabolites for the main groups of methanogens, of H_2/CO_2 for the hydrogenotrophs, and of acetate for aceticlastic methanogens. The identities of all the bacteria involved in these syntrophic relationships aren't known, and the dynamics of these relationships can be complicated, with various microbial groups involved [52,54].

In general, shifts in bacterial communities seem to correlate with changing environmental factors, as the functional component of the community adjusts to take advantage of the available resources and conditions. However, it is unclear if the functional groups in this system do indeed work together as evolutionary partners, or if competition drives their adaptations. To the best of our knowledge, no phylogenetic analysis on the co-evolution of functional microbial groups in lake sediments has been performed.

Deep sediments and vertical LSM profiles:

Isotopic analyses have shown that methane is an important source of C for sediment microbes [18]. This suggests an internal cycling of methane occurs within lakes, and could be an important mechanism of LSM as microbes recycle carbon from the lake ecosystem, including CH_4 produced by the slow metabolism of recalcitrant OM. Several studies looking at vertical profiles of lake sediments and examining the changing archaeal and bacterial communities support this hypothesis [55–59]. O_2 concentration is another factor governing what metabolic pathways are utilized, as when O_2 is depleted, microbes must use alternative electron acceptors and sources of energy to survive. These anaerobic processes and the microbial communities behind them are not well understood, yet form a link in the global carbon cycle.

There is clear evidence that the abundance and composition of the vertical LSM profile is intrinsically linked to the physicochemical characteristics of the lake. For example, there is a general rule that microbial biomass decreases with depth in stratified lake sediments [56,57,60–63]. However, exceptions exist: in Lake Pavin (France), which is permanently stratified with a 30m anoxic monimolimnion, the steady state of the above waters is hypothesized to contribute to a constant profile of microbial abundance in the sediments [55]. There was however an observed shift in archaeal community composition, consistent with other research [55–59]. Typically, in the top layer (~0-5cm depth) of the sediment, the archaeal community is dominated by *Methanosaetacae*. There is then a shift in archaeal taxonomic composition in the middle transition layer (5-25cm depth), and another shift in the microbiome at >25-38 cm depth. One study from Lake Pavin that reported distinct layers, reported this middle layer to have higher proportions of the Marine benthic group D (MBG-D), Miscellaneous Crenarchaeotal Group (MCG), and other *Crenarchaeota* [55]. The lowest layer examined was largely dominated by the

MBG-D group, a taxon that has been found to often dominate the marine subsurface [64]. It is clear however that the deep sediments are a low energy environment, and that, with increasing depth, microbial communities are less affected by pulses of higher quality OM from seasonal fluxes (e.g. algal blooms). It is also clear from the literature that the vertical profile of a lake sediment does not represent one homogenous habitat, but as many as three distinct zones characterized by the physicochemical properties that allow the community to thrive. Additionally, while certain abiotic variables provide indications as to what drives microbial diversity and abundance, these relationships are still poorly understood. Studies have demonstrated that *Euryarchaeota* or *Crenarchaeota* are usually the dominant archaeal taxa in different sediment types however the drivers of Archaeal taxonomic differences remain unclear. Additionally, these studies use broad taxonomic groupings and therefore these studies, particularly those who employ low resolution methods (i.e. TRFLP, DGGE and non-sequencing methods), that are unable to fully assess the diversity of a system and link taxonomic data to important metabolic processes in the system.

Concluding remarks:

It is now necessary to employ high resolution technologies, such as next generation sequencing based methods like metagenomics, metatranscriptomics and metaproteomics, in order to perform a comprehensive survey of these communities. Studies using lower powered DNA fingerprinting techniques leave gaps in our knowledge, as they capture a smaller overview of a LSM. In addition to this, NGS data is meaningless without connection to functional and ecological relevant data. Many important OTUs remain unknown as they have not been isolated in culture, whereupon they can be experimented with and have their genomes analyzed. Until

these microbes are better characterized, the ecological functioning of a LSM will remain unknown, as will the lifestyles of many microbes within lake sediments.

This knowledge is crucial if we are to understand the various micro-habitats created in a lake's sediments through physicochemical factors. As demonstrated in the literature, different zones of a lake can have different LSMs which affect the decomposition of organic matter and the fate of carbon (Fig. 1). This is important as with climate change and warming temperatures the metabolic rates of ecosystems and the balance of sinks and sources of greenhouse gases will change [66]. Therefore, it becomes incredibly important to uncover the mysteries of the microbes in lake sediments, in order to gain a better picture of aquatic ecosystem functioning.

References:

1. Cole, J. J. *et al.* Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. *Ecosystems* **10,** 172–185 (2007).

2. Sundquist, E. T. The global carbon dioxide budget. *Science (80-.).* **259,** 934–941 (1993).

3. Duarte, C. M. & Prairie, Y. T. Prevalence of heterotrophy and atmospheric CO2 emissions from aquatic ecosystems. *Ecosystems* **8,** 862–870 (2005).

4. Jansson, M., Bergström, A.-K., Blomqvist, P. & Drakare, S. Allochthonous organic carbon and phytoplankton / bacterioplankton produciton relationships in lakes. *Ecology* **81,** 3250–3255 (2000).

5. Del Giorgio, P. a, Cole, J. J., Caraco, N. F. & Peters, R. H. Linking Planktonic Biomass and Metabolism to Net Gas Fluxes in Northern Temperate Lakes. *Ecol. Soc. Am.* **80,** 1422–1431 (1999).

6. Tranvik, L. J. *et al.* Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol. Oceanogr.* **54,** 2298–2314 (2009).

7. Bastviken, D. *et al.* Freshwater Methane Emissions Offset the Continental Carbon Sink. *Science (80-.).* **331,** 50 (2011).

8. Downing, J. A. *et al.* The global abundance and size distribution of lakes , ponds , and impoundments. *Limnol. Oceanogr.* **51,** 2388–2397 (2006).

9. Gudasz, C. *et al.* Temperature-controlled organic carbon mineralization in lake sediments. *Nature* **466,** 478–481 (2010).

10. Dean, W. E. & Gorham, E. Magnitude and Signifigance of Carbon Burial in Lakes, Reservoirs, and Peatlands. *Geology* **26,** 535–538 (1998).

11. Dossena, M. *et al.* Warming alters community size structure and ecosystem functioning. *Proc. R. Soc. B Biol. Sci.* **279,** 3011–3019 (2012).

12. Einsele, G., Yan, J. & Hinderer, M. Atmospheric carbon burial in modern lake basins and its significance for the global carbon budget. *Glob. Planet. Change* **30,** 167–195 (2001).

13. Kritzberg, E. S. *et al.* Autochthonous versus allochthonous carbon sources of bacteria : Results from whole-lake 13C addition experiments. *Limnol. Oceanogr.* **49,** 588–596 (2004).

14. Meyers, P. A. & Ishiwatari, R. Lacustrine organic geochemistry-an overview of indicators of organic matter sources and diagenesis in lake sediments. *Org. Geochem.* **20,** 867–900 (1993).

15. West, W. E., Coloso, J. J. & Jones, S. E. Effects of algal and terrestrial carbon on methane production rates and methanogen community structure in a temperate lake sediment. *Freshw. Biol.* **57,** 949–955 (2012).

16. Bergstrom, A. K. & Jansson, M. Bacterioplankton Production in Humic Lake Ortrasket in Relation to Input of Bacterial Cells and Input of Allochthonous Organic Carbon. *Microb. Ecol.* **39,** 101–115 (2000).

17. Hanlon, R. D. G. Allochthonous plant litter as a source of organic material in an oligotrophic lake (Llyn Frongoch). *Hydrobiologia* **80,** 257–261 (1981).

18. Steger, K. *et al.* Comparative study on bacterial carbon sources in lake sediments: the role of methanotrophy. *Aquat. Microb. Ecol.* **76,** 39–47 (2015).

19. Bastviken, D., Cole, J. J., Pace, M. L. & Van de Bogert, M. C. Fates of methane from different lake habitats: Connecting whole-lake budgets and CH_4 emissions. *J.*

Geophys. Res. **113,** G02024 (2008).

20. Battin, T. J. *et al.* The boundless carbon cycle. *Nat. Geosci.* **2,** 598–600 (2009).

21. Belle, S., Verneaux, V., Millet, L., Parent, C. & Magny, M. A case study of the past CH4 cycle in lakes by the combined use of dual isotopes (carbon and hydrogen) and ancient DNA of methane-oxidizing bacteria: rearing experiment and application to Lake Remoray (eastern France). *Aquat. Ecol.* 279–291 (2015). doi:10.1007/s10452-015-9523-6

22. Bastviken, D., Cole, J., Pace, M. & Tranvik, L. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochem. Cycles* **18,** 1–12 (2004).

23. Conrad, R. The global methane cycle: Recent advances in understanding the microbial processes involved. *Environ. Microbiol. Rep.* **1,** 285–292 (2009).

24. Cui, M., Ma, A., Qi, H., Zhuang, X. & Zhuang, G. Anaerobic oxidation of methane: An 'active' microbial process. *Microbiologyopen* **4,** 1–11 (2015).

25. Grey, J. The incredible lightness of being methane-fueled stable isotopes reveal alternative energy pathways in aquatic ecosystems and beyond. *Front. Ecol. Evolition* **4,** 1–14 (2016).

26. Borrel, G. *et al.* Production and consumption of methane in freshwater lake ecosystems. *Res. Microbiol.* **162,** 833–847 (2011).

27. Gudasz, C., Bastviken, D., Premke, K., Steger, K. & Tranvik, L. J. Constrained microbial processing of allochthonous organic carbon in boreal lake sediments. *Limnol. Oceanogr.* **57,** 163–175 (2012).

28. Premke, K. *et al.* Stable isotope analysis of benthic fauna and their food sources in boreal lakes. *J. North Am. Benthol. Soc.* **29,** 1339–1348 (2010).

29. Solomon, C. T., Carpenter, S. R., Cole, J. J. & Pace, M. L. Support of benthic invertebrates by detrital resources and current autochthonous primary production: Results from a whole-lake 13C addition. *Freshw. Biol.* **53,** 42–54 (2008).

30. Harrault, L. *et al.* Bottom-up effects of lake sediment on pelagic food-web compartments: A mesocosm study. *Freshw. Biol.* **59,** 1695–1709 (2014).

31. Eller, G., Deines, P., Grey, J., Richnow, H. H. & Kruger, M. Methane cycling in lake sediments and its influence on chironomid larval 13C. *FEMS Microbiol. Ecol.* **54,** 339–350 (2005).

32. Grey, J., Kelly, A. & Jones, R. I. High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. *Limnol. Oceanogr. [Limnol. Ocean.* **49,** 239–244 (2004).

33. Kelly, A., Jones, R. I. & Grey, J. Stable isotope analysis provides fresh insights into dietary separation between Chironomus anthracinus and C. plumosus. *J. North Am. Benthol. Soc.* **23,** 287–296 (2004).

34. Kiyashko, S. I., Narita, T. & Wada, E. Contribution of methanotrophs to freshwater macrioinvertebrates: evidence from stable isotope ratios. *Aquat. Microb. Ecol.* **24,** 203–207 (2001).

35. Bunn, S. E. & Boon, P. I. What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. *Oecologia* **96,** 85–94 (1993).

36. Heyer, C. den & Kalff, J. Organic matter mineralization rates in sediments: A within- and among-lake study. *Limnol. Oceanogr.* **43,** 695–705 (1998).

37. Prairie, Y. T. & del Giorgio, P. A. A new pathway of freshwater methane emissions and the putative importance of microbubbles. *Inl. Waters* **3,** 311–320 (2013).

38. Hofmann, H., Federwisch, L. & Peeters, F. Wave-induced release of methane : Littoral zones as a source of methane in lakes. *Limnol. Oceanogr.* **55,** 1990–2000 (2010).

39. Tšertova, N., Kisand, A., Baty, F. & Kisand, V. Homogeneous microbial diversity in the upper sediment layers of a shallow lake. *Aquat. Microb. Ecol.* **70,** 77–85 (2013).

40. Wurzbacher, C. M., Barlocher, F. & Grossart, H. P. Fungi in lake ecosystems. *Aquat. Microb. Ecol.* **59,** 125–149 (2010).

41. Bärlocher, F. Aquatic fungal ecology. *Fungal Ecol.* **19,** 1–4 (2016).

42. Nagano, Y. *et al.* Fungal diversity in deep-sea sediments - the presence of novel fungal groups. *Fungal Ecol.* **3,** 316–325 (2010).

43. Bärlocher, F. Aquatic hyphomycetes in a changing environment. *Fungal Ecol.* **19,** 14–27 (2015).

44. Zhang, J. *et al.* Distribution of sediment bacterial and archaeal communities in plateau freshwater lakes. *Appl. Microbiol. Biotechnol.* **99,** 3291–3302 (2015).

45. Song, H., Li, Z., Du, B., Wang, G. & Ding, Y. Bacterial communities in sediments of the shallow Lake Dongping in China. *J. Appl. Microbiol.* **112,** 79–89 (2012).

46. Logue, J. B. *et al.* Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *ISME J.* 1–13 (2015). doi:10.1038/ismej.2015.131

47. Bouzat, J. L., Hoostal, M. J. & Looft, T. Spatial patterns of bacterial community composition within Lake Erie sediments. *J. Great Lakes Res.* **39,** 344–351 (2013).

48. Hoostal, M. J. & Bouzat, J. L. The modulating role of dissolved organic matter on spatial patterns of microbial metabolism in Lake Erie sediments. *Microb. Ecol.* **55,** 358–368 (2008).

49. Chen, N. *et al.* Sediment prokaryote communities in different sites of eutrophic Lake Taihu and their interactions with environmental factors. *World J. Microbiol. Biotechnol.* **31,** 883–896 (2015).

50. Tsertova, N., Kisand, A., Tammert, H. & Kisand, V. Low seasonal variability in community composition of sediment bacteria in large and shallow lake. *Environ. Microbiol. Rep.* **3,** 270–277 (2011).

51. Swan, B. K., Ehrhardt, C. J., Reifel, K. M., Moreno, L. I. & Valentine, D. L. Archaeal and bacterial communities respond differently to environmental gradients in anoxic sediments of a california hypersaline lake, the Salton Sea. *Appl. Environ. Microbiol.* **76,** 757–768 (2010).

52. Mcinerney, M. J., Sieber, J. R. & Gunsalus, R. P. Syntrophy in anaerobic global carbon cycles. *Curr. Opionion Biotechnol.* 623–632 (2009). doi:10.1016/j.copbio.2009.10.001

53. Müller, N., Griffin, B. M., Stingl, U. & Schink, B. Dominant sugar utilizers in sediment of Lake Constance depend on syntrophic cooperation with methanogenic partner organisms. *Environ. Microbiol.* **10,** 1501–1511 (2008).

54. Hattori, S. Syntrophic acetate-oxidizing microbes in methanogenic environments. *Microbes Environ.* **23,** 118–127 (2008).

55. Borrel, G. *et al.* Stratification of Archaea in the deep sediments of a freshwater meromictic lake: Vertical shift from methanogenic to uncultured Archaeal lineages. *PLoS One* **7,** (2012).

56. Chim Chan, O. *et al.* Vertical distribution of structure and function of the methanogenic archaeal community in Lake Dagow sediment. *Environ. Microbiol.* **7,**

1139–1149 (2005).

57. Koizumi, Y., Takii, S., Nishino, M. & Nakajima, T. Vertical distributions of sulfate-reducing bacteria and methane-producing archaea quantified by oligonucleotide probe hybridization in the profundal sediment of a mesotrophic lake. *FEMS Microbiol. Ecol.* **44,** 101–108 (2003).

58. Liu, L., Peng, Y., Zheng, X., Xiaoa, L. & Yang, L. Vertical Structure of Bacterial and Archaeal Communities within the Sediment of a Eutrophic Lake as Revealed by Culture-Independent Methods. *J. Freshw. Ecol.* **25,** 565–573 (2010).

59. Tsutsumi, M., Kojima, H. & Fukui, M. Vertical Profiles of Abundance and Potential Activity of Methane-Oxidizing Bacteria in Sediment of Lake Biwa, Japan. *Microbes Environ.* **27,** 67–71 (2012).

60. Rothfuss, F., Bender, M. & Conrad, R. Survival and activity of bacteria in a deep, aged lake sediment (Lake Constance). *Microb. Ecol.* **33,** 69–77 (1997).

61. Miskin, I., Rhodes, G., Lawlor, K., Saunders, J. R. & Pickup, R. W. Bacteria in post glacial freshwater sediments. *Microbiology* **144,** 2427–2439 (1998).

62. Zepp Falz, K. *et al.* Vertical distribution of methanogens in the anoxic sediment of Rotsee (Switzerland). *Appl. Environ. Microbiol.* **65,** 2402–2408 (1999).

63. Haglund, A. L., Lantz, P., Törnblom, E. & Tranvik, L. Depth distribution of active bacteria and bacterial activity in lake sediment. *FEMS Microbiol. Ecol.* **46,** 31–38 (2003).

64. Lloyd, K. G. *et al.* Predominant archaea in marine sediments degrade detrital proteins. *Nature* **496,** 215–8 (2013).

65. Lucheta, A. R., Otero, X. L., Macías, F. & Lambais, M. R. Bacterial and archaeal

communities in the acid pit lake sediments of a chalcopyrite mine. *Extremophiles* **17,** 941–951 (2013).

66. Yvon-Durocher, G., Jones, J. I., Trimmer, M., Woodward, G. & Montoya, J. M. Warming alters the metabolic balance of ecosystems. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **365,** 2117–2126 (2010).

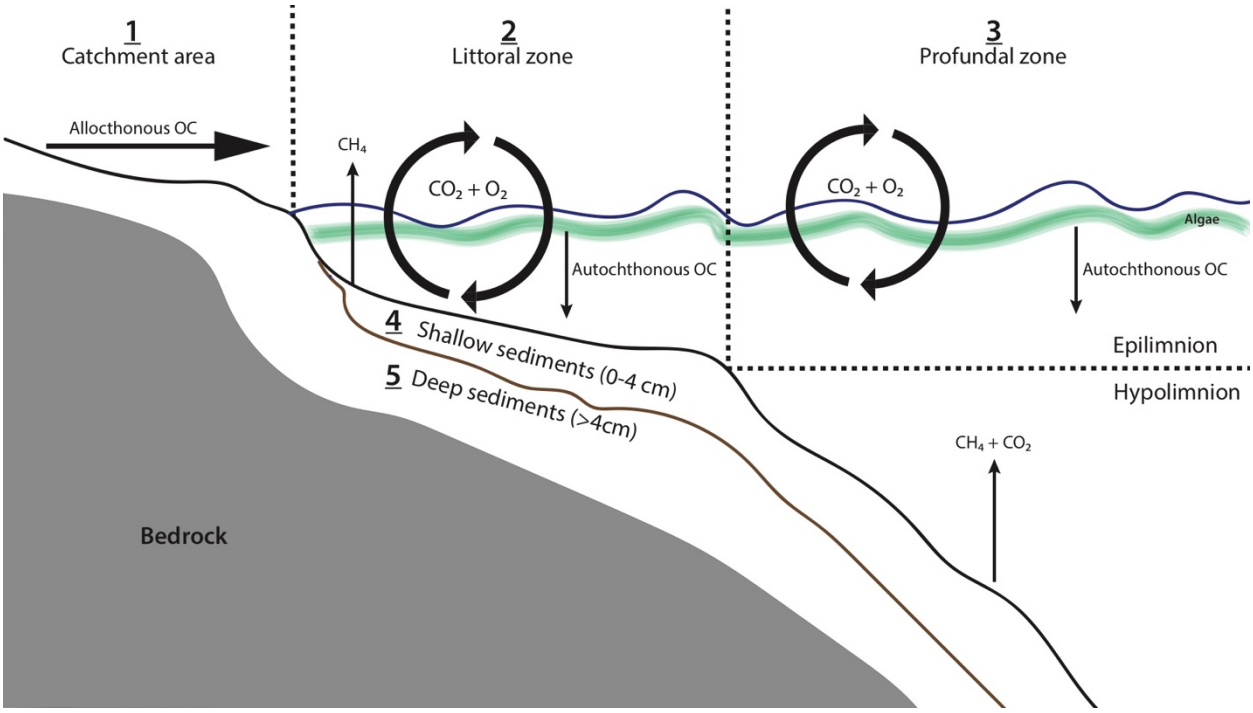


Figure 1.1: A general model of the carbon cycle in lakes, emphasizing different zones that have unique microbial habitats.

Chapter 2: Plant litter type dictates microbial communities responsible for greenhouse gas production in lake sediments

Kurt M. Yakimovich[1,3*], Erik J. Szkokan-Emilson[4], Michael A. Carson[1,3], Andrew J. Tanentzap[4], Nathan Basiliko[1,3] and Nadia C.S. Mykytczuk[1,2,3,]

[1] Vale Living with Lakes Centre, Laurentian University, Sudbury ON, Canada
[2] School of the Environment, Laurentian University, Sudbury ON, Canada
[3] Department of Biology, Laurentian University, Sudbury ON, Canada
[4] Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom

Abstract:

The microbial communities of lake sediments play key roles in the cycling of C; linking lakes to both surrounding landscapes that are sources of organic inputs and to the global climate system via greenhouse gas emissions. Here we amended lake sediments with three different plant leaf litters: a coniferous forest mix, deciduous forest mix and cattails (*Typha latifolia*) and examined the community structure and numbers of bacteria, fungi and methanogens. We found polyphenol levels that varied with the different litters selected for unique bacterial, methanogen and fungal communities, including dominance of fungi over bacteria as polyphenol levels increased resulting from increases of the white rot fungi *Phlebia* spp. Additionally, we saw a shift in the dominant orders of fermentative bacteria with increasing polyphenol levels and differences in the dominant methanogen groups, with high CH_4 production being more strongly associated with generalist groups of methanogens found at lower polyphenol levels. This study provides insight into how shifting upland and wetland plant communities may influence anaerobic microbial communities and processes in lake sediments, and may alter the fate of terrestrial C entering inland waters.

Introduction:

Freshwater ecosystems have an important role in the global carbon (C) cycle because they can offset the terrestrial C sink by 79% via CO_2 and CH_4 emissions (Bastviken *et al.*, 2011). A large portion of the C cycled through freshwater ecosystems comes from plants, with an estimated 2.9 Pg of terrestrial C $year^{-1}$ entering freshwaters from terrestrial systems (3 times what oceans receive) (Tranvik *et al.*, 2009). Earth's ~304 million lakes are central to C-cycling, harboring sediment microbial communities responsible for decomposing and mineralizing

terrestrially derived C that is in the form of organic matter (OM) (Downing *et al.*, 2006). Most lakes are shallow with organic-rich sediments that support obligately anaerobic CH_4 production. Evasion of CH_4 to the atmosphere can be accelerated by processes such as plant mediated efflux, ebullition, and wave action disturbance of the sediments (Hofmann *et al.*, 2010), which highlights the importance of shallow sediments for overall lake CH_4 budgeting (Bastviken *et al.*, 2008). Additionally, with CH_4 being ~25 times more potent a greenhouse gas than CO_2 over a 100 yr timeframe, it becomes important to understand how microbial decomposers direct terrestrial C into CH_4 (Forster *et al.*, 2007).

Terrestrially-derived C differs in biogeochemical composition from within-lake sources of OM (Wetzel, 1992; Ye *et al.*, 2016; Field *et al.*, 1988), however, little is known about how these differences influence sediment microbial decomposer communities (Logue *et al.*, 2015; Bouzat *et al.*, 2013; Hoostal and Bouzat, 2008; Chen *et al.*, 2015). While autochthonous sources of OM are preferentially degraded and readily mineralized into CH_4 (West *et al.*, 2012, 2015), the vast majority (87%; Kritzberg *et al.,* 2004) of dissolved organic C (DOC) in lakes is from terrestrial sources and is considered recalcitrant (Kritzberg *et al.,* 2004). Recalcitrance has partly been linked to increasing abundance of polyphenolic compounds, which have been shown to inhibit extracellular bacterial enzymes involved in decomposition and have a toxic on methanogens (Wetzel, 1992; Ye *et al.*, 2016; Field *et al.*, 1988), but little literature exists exploring the effects of OM inputs from shifting plant communities on bacteria or methanogens in lake sediments. An even larger gap exists when it comes to exploring fungi in lake sediments, as research has largely focused on the syntrophy of bacteria and methanogens (e.g. Bastviken *et al.*, 2008; Mcinerney *et al.*, 2009). Despite some evidence that fungi are important decomposers in other anaerobic systems (e.g. Brad *et al.*, 2008), little effort has been given to examine their

role in lake sediments (Wurzbacher *et al.*, 2010). Therefore, widespread changes in plant community composition in catchments of freshwater systems—resulting from different land use practices and/or climate change (Millar *et al.*, 2007; Foley *et al.*, 2005)—have the potential to shift lake sediment bacterial, fungal and methanogen communities and subsequently impact lake-level C emissions.

Previously, we found that the decomposition of plant litter from the emergent macrophyte *Typha latifolia* released at least 400X more CH_4 than that of terrestrial plant litters (Szkokan-Emilson *et al.*, 2017). *T. latifolia* loading in sediments also increased methanogen abundance in contrast to forest litters with high polyphenol levels, which appeared to supress methanogenesis and methanogen cell propagation (Szkokan-Emilson *et al.*, 2017). Given that climate change is expected to increase emergent macrophyte coverage in temperate and boreal lakes (Jeppesen et al., 2009; Alahuhta et al., 2011), these results warrant examination of how shifting plant communities and litter types influence microbial communities in lake sediments. We now build on these findings by asking: (1) Do bacterial, fungal and methanogen communities vary in composition and diversity with different plant litters? (2) Do polyphenol levels across plant litters influence dominant bacterial, fungal or methanogen taxa? (3) Are fungi important anaerobic decomposers of plant litters in lake sediments? (4) Do sediments containing a higher OM concentration have a different response to plant litter amendments than low OM content sediments? To address our questions, we analysed lake sediment microbial community responses in-vitro to amendments with different plant litters using two different starting sediments.

Methods:

Experimental design

This work relied on the experimental setup described in Szkokan-Emilson *et al.* (2017). Briefly, sediments for anaerobic incubations were constructed by amending low OM content (0.3% dry weight) lake sediments collected from the top 5 cm of Geneva Lake, Ontario Canada (46°45'27.2"N, 81°33'19.8"W). Amendments consisted of a boreal species coniferous needle mix (CON; *Pinus resinosa* and *P. strobus*), deciduous leaf mix (DEC; *Acer rubrum*, *A. saccharum*, *Betula* spp., *Populus tremuloides*, *Ulmus americanum*, *Quercus rubra*, and *Q. alba*), and the emergent macrophyte *Typha latifolia* (TYP)—amended treatments are hereafter referred to by their respective abbreviations. Each organic matter type was incorporated into the natural lake sediment at either a 10, 20, or 40% dry weight concentration. Sediments were placed in 250 mL mason jars then saturated with TOC-scrubbed A10 MilliQ water (EMD Millipore Corp., Darmstadt, Germany). The jars were then sealed with a lid fitted with a rubber septa and headspace air was removed and replaced with N_2 to create anaerobic conditions. We constructed a second set of the same amendments as described above but with a "spike" of 5% by dry weight of sediments from Ramsey Lake ON (46°28'19.8"N, 80°58'19.2"W) which contained 3% by dry weight OM. OM content of each sediment was obtained by oven drying subsamples at 60 °C to get a dry weight conversion and subsequently combusted at 500 °C for two hours to calculate OM%. All sediments were incubated at 20.5°C for 150 days. During trial experiments of our setup, the maintenance of anoxic conditions was confirmed throughout incubation periods via Oxoid Anaerobic Indicator strips (Thermo Fisher Scientific).

At the end of the incubation, we characterized microbial communities by extracting sediment DNA and related community composition and abundance to physicochemical measurements taken on the same samples by Szkokan-Emilson *et al.*, (2017). Physicochemical data included periodic measurements of CO_2 and CH_4 concentrations by gas chromatography (SRI 8610C with a 0.5mL sample loop and 105°C), and the total concentration and chemical characteristics of dissolved organic matter at the end of the experiment. Post-incubation pore water pH measurements were taken using a HI 9126 pH meter (Hanna Instruments). Dissolved organic matter fluorescence excitation-emission matrices (EEMs) were generated from the pore water of each sample using an Agilent Cary Eclipse Fluorescence Spectrophotometer in ratio (S/R) mode with a 1-cm path length cuvette, with EEMs generated from excitation and emission intensities. EEMs were adjusted for inner-filter effects by absorbance with measurements from an Agilent Cary 60 UV-Vis Spectrophotometer. A total of five components were identified via PARAFRAC modelling (referred to as C1 to C5 defined by maximum excitation/emission intensities). Briefly there were humic-like components C1 (310/414nm) and C2 (345/462nm), a tryptophan-like protein component C3 (280/354nm), a tyrosine-like protein components C4 (270/306nm), and to a protein-like polyphenol component C5 (275/318nm) (Yamashita *et al.*, 2008; Maie *et al.*, 2008, 2007).

DNA extractions

Sediments were sub-sampled from the jars and immediately frozen and stored at -20°C for microbial community analysis. Each quadruplicate sample was thawed and DNA was extracted in duplicate using the MoBio PowerSoil kit (Mo Bio, Carlsbad, CA, U.S.A.). Pairs from the quadruplicate extracts were combined, resulting in duplicate DNA samples representing

each treatment (each constituting 4 DNA extractions). DNA was concentrated down into 40 µL aliquots suspended in the C6 solution from the MoBio PowerSoil kit (Mo Bio). Sample DNA was quantified using a Take3 spectrophotometry system on a Synergy HI microplate reader (BioTek, Winooski VT, USA).

Amplicon sequencing

Sequencing of duplicate DNA samples representing each treatment was carried out on an Illumina MiSeq system (Illumina Biotechnology Co., San Diego, USA) by Metagenome Bio Inc. (Toronto, Canada), with three targets: general prokaryote primers to target bacterial 16S rRNA genes Pro341F (5'-CCT ACG GGN BGC ASC AG-3') and Pro805R (5'-GAC TAC NVG GGT ATC TAA TCC-3) (Takahashi *et al.*, 2014); *mcrA* primers to target methanogens mlas (5'-TGG YGG TGG TMG GDT TCA CMC ART A-3') and mcrA R (5'-CGT TCA TBG CGT AGT TVG GRT AGT-3) (Angel *et al.*, 2011); and ITS primers to target fungi ITS1F (5'-ACC TGC GGA RGG ATC A-3') and ITS1R (5'-GAG ATC CRT TGY TRA AAG TT-3') (Bokulich and Mills, 2013). Samples had an index sequence (6 bp) incorporated before being pooled to be sequenced in one run. A 25 µl PCR reaction was performed containing 5 µl of standard OneTaq buffer (5x), 0.25 µl of 25mM dNTP, 0.5 µl of both the forward and reverse primers, 1 µl BSA (12 mg/ml), 0.125 µl of OneTaq DNA polymerase (New England Bio, MA, U.S.A.), 1-10 ng DNA and water to equal a total reaction volume of 25 µl. Initial denaturation started at 94^{o}C for 5 mins followed by 30 cycles of 94 ^{o}C for 30 sec denaturation, annealing was at 53 ^{o}C for 16s, 62 ^{o}C for 18s *mcr*A for 45 sec, extension at 68 ^{o}C for 1 min with a final extension step at 68^{o}C for 10 minutes. PCRs were done in triplicate for each sample to reduce PCR bias and products were checked on a 2% agarose gel, after which the bands were excised with a MinElute gel extraction kit (Qiagen,

Hilden, Germany).The purified DNA libraries were quantified on a Quibit with the dsDNA HS assay kit (Life Technologies, CA,USA), the library pools were spiked with 5% phix control (V3, Illumina) to improve base imbalance, and paired-end sequencing with read lengths of 250 bp was performed using MiSeq Reagent kit V2 (2 x 250 cycles) on a Illumina MiSeq platform.

qPCR

Quantitative PCR (qPCR) was carried out in triplicate on the total pooled DNA of each experimental treatment to determine relative abundance of bacteria, fungi and methanogens. qPCR has been shown to be a reliable metric to compare relative abundance of fungi and bacteria and generally mirrors biomass dynamics (Rousk et al., 2010; Fierer et al., 2005). Primers targeted: 16S rRNA gene Eub-338 (5'- GCT GCC TCC CGT AGG AGT-3') and Eub 518 (5'-ATT ACC GCG GCT GCT GG-3') for bacteria (Fierer *et al.*, 2005); and 18S rRNA gene, FU18S1 (5'-GGA AAC TCA CCA GGT CCA GA-3') and Nu-SSU-1536 (5'-ATT GCA ATG CYC TAT CCC CA-3') for fungi (Gangneux *et al.*, 2011) and the same primers were used *mcr*A sequencing above were used for methanogen qPCR. The bacterial and fungal reaction conditions (16S/*mcr*A/18S genes respectively) were a 5-minute initial denaturation at 95°C, followed by 30/35 cycles of 95°C for 10 sec, 53/55/62°C for 10 seconds, and 72°C for 10 seconds with a final denaturation for 1 minute at 95°C. The qPCR was done using Biorad's iTaq universal Sybr green Supermix on an Agilent Technologies Stratagene Mx3005P. Copy numbers for bacteria and fungi were calculated using standard curves of *Escherichia coli* (ATCC#11303) and *Saccharomyces cerevisiae* (ATCC#2360), giving final units of copies per gram of dry weight of sediment. A standard curve was made for *mcr*A using a serially diluted purified PCR product of amplified DNA which was run in triplicate, and values were standardized relative to the control

and per gram of dry weight. All qPCR runs had R^2 values greater than 99% and efficiency values greater than 90%. Final product purity was confirmed via dissociation curve and on a 1.5% agarose gel.

Data analysis

Raw Miseq reads were merged using PANDAseq for the *mcr*A and 16S rRNA gene reads, and using PEAR for the fungal ITS fragments (Stamatakis *et al.*, 2014; Masella *et al.*, 2012). All reads were then further quality filtered using USEARCH v8.1.1861, and taxonomy was assigned using QIIME with the Green Genes database (DeSantis *et al.*, 2006) for 16S rRNA gene reads, the UNITE database (Koljalg *et al.*, 2013) for ITS fungal reads, and a database created by Yang *et. al.* (2014) for *mcr*A reads. Data were then imported and analyzed in R using the phyloseq package (R Core Team, 2016; McMurdie & Holmes, 2013). Abundance data from sequencing used was first normalized using the DeSEq2 package in R (Love *et al.*, 2014). The sequencing data has been deposited into NCBI under BioProject PRJNA347436, containing SRA samples SRR4418117-SRR4418160.

Phylogenetic trees were constructed for UniFrac (distance matrices that incorporate phylogenetic distances) analysis from the global data sets of bacteria, fungi and methanogens and a subset of bacterial OTUs with relative abundance >0.01%. Sequences were first aligned using MUSCLE and the resulting alignments manually trimmed in MEGA7 (Edgar, 2004; Kumar *et al.*, 2016). Maximum likelihood trees were then constructed using RAxML, with 1000 bootstrap replicates (Stamatakis, 2014). A second tree was constructed for the methanogen OTUs that included representatives of 6 methanogen Orders, sequences were obtained from NCBI's GenBank (Clark *et al.*, 2016). The resulting tree then had branches grouped and highlighted by

Order to display phylogenetic diversity of obtained OTUs. To further examine microbial diversity patterns, the Chao 1 index was calculated with the Fossil package (Vavrek, 2011) in R using normalized read counts with singletons and doubletons removed.

Gas, pH, qPCR, DOM data and relative abundance of OTUs determined from sequence libraries were analyzed for significant relationships using Pearson's product moment correlations, one- and two-way analysis of variance (ANOVA) where appropriate, and a Permutational Multivariate Analysis of Variance using Distance Matrices (ADONIS) using core R and the vegan package (R Core Team, 2016; Oksanen *et al.*, 2017). Indicator species analyses were performed using the Indicspecies multipatt function in R (De Caceres and Legendre, 2009) and the gplots Heatmap.2 function (Warnes *et al.*, 2016). UniFrac distances, both unweighted (presence absence of OTUs) and weighted (uses relative abundances of OTUs) and PCoA plot construction was done using the Phyloseq R package (McMurdie and Holmes, 2013). To analyze the top most abundant bacterial taxa, sample relative abundances were plotted across samples from DEC (containing the highest measured polyphenols) then sequentially CON and TYP (containing lower polyphenol levels) and ordering each plant litter's samples from 40% to 10% of both un-spiked and spiked treatments. This allowed the variation in dominant bacterial taxa at various taxonomic levels to be visualised across a pseudo-polyphenol concentration gradient.

Results

We found that bacterial, fungal and methanogen communities in the litter amended sediments were all different from unamended controls (Fig. 1). The composition of bacterial and methanogen communities varied significantly across litter types and concentrations whereas fungi only varied across litter type (ADONIS test for litter type and concentration, respectively:

bacteria—$p < 0.001$ and $p < 0.001$; fungi—$p < 0.001$ and $p = 0.242$; methanogens—$p < 0.001$, $p = 0.041$). The ADONIS also revealed that litter type explained more of the variation in the bacterial and methanogen communities (litter type and concentration, respectively, for: bacteria $R^2 = 0.52$, $R^2 = 0.04$; methanogens $R^2 = 0.42$, $R^2 = 0.04$), therefore we grouped samples by litter type alone in Fig. 1. PCoA ordination of unweighted UniFrac distance matrices further revealed that bacterial and fungal communities in the CON and DEC samples were different from those in the CTR and TYP amendments, but shared some 95% confidence interval overlap (Fig. 1A&B). The methanogen communities showed less heterogeneity in the both the CON and TYP treatments comparted to the DEC (Fig. 1C). Looking at the un-amended controls, we observed homogeneity in all three microbial communities and they remained similar to the starting sediments after 150 days of incubation (Fig.1).

Community structure and diversity

All three organic matter additions resulted in unique bacterial communities in contrast to the starting sediments, but more similarities existed between CON communities and DEC communities than either with the TYP communities (Fig. 1A; unweighted UniFrac). When bacterial relative abundances were considered, TYP and CON treatments were more similar (Fig. S1; weighted UniFrac). In all cases, the plant litter amendments resulted in lower bacterial Chao 1 diversity values by 17- 59% (Fig 2A). Even with this reduction in bacterial diversity across plant litter treatments, we did not observe any distinct indicator OTUs for specific plant litter types.

The fungal communities were largely unique across plant litter types, with the different litters resulting in different compositions compared to those in the pre-incubation sediments and

leaf litter material, except for the DEC treatment (Fig.1B). Although there was a slight overlap of the confidence intervals between the two tree leaf litters, differences between the communities were strongly dictated by litter type (Fig. 1B). Plant litter amendments also resulted in an increase in fungal diversity reflected by Chao 1 by 23 - 86% (Fig. 2B). From the communities for each litter type a total of 49 indicator OTUs were identified, with a majority identifiable at the family and genus levels (Fig. 3). These indicators were part of the subset of the 131 of 4196 most abundant fungal OTUs (>1% within at least one sample).

The methanogen communities in the DEC and CON treatments were dominated by OTUs unidentifiable at the order level with some Methanobacteriales, Methanosarcinales, and Methanomassilicoccus and no distinct indicator OTUs (all methanogen OTUs displayed in Fig. 4). Across all plant litters, we found an increase in methanogen Chao 1 diversity relative to un-incubated plant litters (Fig. 2C), with both DEC and TYP plant litters having no detectable methanogen OTUs and CON having only 1. Overall Chao 1 values for the methanogens increased to between 3 and 17% in the litter amended sediments. The TYP 10 and 20% OM samples were primarily composed of two orders of methanogens, Methanosarcinales (OTU 2) and Methanobacteriales (OTU 1), whereas both un-spiked and spiked TYP 40% OM samples were dominated by Methanobacteriales (Fig. 4). This shift in the dominant methanogen taxa was linked to the total amount of CH_4, as members of the methanogen family Methanosarcinaceae were the only group that were positively correlated with total CH_4 production across 150 days ($r = 0.49$, $p < 0.005$). Additionally, the TYP treatments started producing CH_4 in order of increasing OM concentration (10% started producing on day 31, 20% on day 60 and 40% on day 91), with 10% having produced the most by day 150, then the 20% and 40 % treatments (Fig. S5).

Microbial associations with DOM

Variation in the bacterial communities was related to relative polyphenol concentration across amendments (Fig 5). The un-spiked and spiked treatments were mostly similar across litter amendments except for between the CON 20% and 40% OM treatments, where Bacteroidales were only found in the spiked samples. An additional difference was between the highest polyphenol containing samples of DEC litter with a 40% OM concentration. The spiked DEC 40% OM treatment was dominated by Rhodospirillales (comprised of *Acetobacter* spp. OTUs) and Lactobacillales (largely comprised of *Leuconostoc* spp. and OTUs unidentifiable at the genus level). The un-spiked DEC 40% OM along with the other DEC concentrations, both spiked and un-spiked, were dominated by OTUs from the order Bacillales (primarily OTU 1, which is a *Sporolactobacillus* sp.). Lower abundance bacterial orders included Enterobacteriales, Coriobacteriales, and OPB54, none of which showed clear patterns across treatments (Fig. S2). Differences in pH were also observed between the spiked DEC (mean = 3.62; SE±0.25) and un-spiked (mean = 4.2; SE±0.1) treatments, and DEC had a lower pH than either the CON or TYP treatments with both un-spiked and spiked sediments (two-way ANOVA: $F_{3,72} = 7.99$, $p < 0.001$, Tukey's: $p < 0.001$ for all comparisons). The differences in pH were also reflected in the control sediments, with the un-spiked control having a mean pH of 6.94 (SE±0.24) and the spiked being 4.93 (SE±0.32).

Bacterial 16S rDNA and methanogen *mcr*A abundances were linked to DOM characteristics and in turn to CO_2 production. Our analysis of DOM characteristics revealed that bacterial and methanogen abundances positively correlated with humic-like components C1 (16S rDNA r = 0.68, p = 0.002; *mcr*A r = 0.72, p < 0.001) and C2 (16S rDNA r = 0.0.68, p = 0.002;

*mcr*A r = 0.69, p = 0.002. Whereas the C4 protein-like component correlated with just bacterial abundance (16S rDNA r = 0.49, p = 0.047). The increase in bacterial and methanogen abundances also corresponded with an increase in CO_2 production (respectively: r= 0.56, p = 0.021; r = 0.96, p < 0.001). This increase of bacterial abundance was associated with TYP amendment (one-way ANOVA: $F_{5,16} = 25.6$, p < 0.001; Tukey's test for all comparisons including TYP: p < 0.001). In general, these patterns revealed how TYP enhanced both bacterial and methanogen activity and cell propagation.

Fungi vs. Bacteria

The ratio of fungi to bacteria was examined to determine how different plant litters affected the dominant decomposers and if there was evidence of competition between them. We found a negative correlation between bacteria and fungi (r = -0.60 p = 0.010: values summarized in Table 1). Further examination revealed that bacterial 16S rDNA copy number and polyphenols negatively correlated (r = -0.73, p < 0.001) while fungal 18S copies positively correlated with polyphenol levels (r = 0.83, p < 0.001). Polyphenol concentrations were different amongst plant litters and were highest in DEC samples (one-way ANOVA, $F_{2,14} = 15.66$, p < 0.001; Tukey's, DEC-CON p = 0.0146, DEC-TYP p = 0.001), which corresponded to a higher copy number of fungi (one-way ANOVA, $F_{2,14} = 16.62$, p < 0.001; Tukey's, DEC-CON p = 0.004, DEC-TYP p < 0.001). Examining the increase in fungal abundance further, we observed a positive correlation with *Phlebia* spp. (r = 0.79, p < 0.001), which had high relative abundances in the DEC amended sediments. These results demonstrated a dominance of fungi over bacteria in the presence of high polyphenol containing litter, even in an anaerobic environment.

Discussion

Here we examined the lake sediment microbial communities involved in decomposing different types of plant litters that we previously showed to have large differences in rates of greenhouse gas production (Szkokan-Emilson *et al.*, 2017). We linked community composition and diversity to biochemical differences created by different litters, which had an environmental filtering effect on the microbial communities. These findings extend the conclusions of previous work linking microbial community dynamics in lake waters to DOM sources (Logue *et al.*, 2016). Our results agree with past literature that emphasizes the importance of the chemical composition of plant litter when evaluating decomposition rates, not simply litter species richness (Lecerf *et al.*, 2007; Schindler and Gessner, 2009; Swan *et al.*, 2009; Wardle *et al.*, 1997); however, our study also extends this paradigm to lake sediments and includes fungi as well as bacteria and methanogens as important decomposers in anaerobic lake sediments. Therefore, our findings have implications for the fate of terrestrial-derived C as they demonstrate how plant litter chemistry selects for different microbial decomposers and subsequently different mineralization rates and products. These differences can alter the residence time of C in aquatic systems as DOM composition influences whether C is incorporated into food webs, buried in sediments or emitted to the atmosphere (Heyer and Kalff, 1998).

Polyphenols had a strong influence on bacterial and fungal and methanogen community structure and abundances of bacteria and fungi. While DEC and CON treatments had similar polyphenol concentrations initially, DEC treatments maintained higher levels throughout the experiment (Table 1), which suggests that microbial communities in the CON treatments could break down the polyphenolic compounds more readily. The faster degradation of polyphenolic

compounds could either be due to the presence of adaptive biochemical pathways or chemical differences in the soluble compounds. Differences in abundances of bacteria and fungi revealed a certain degree of niche partitioning, with fungi showing greater abundance in the DEC treatments than bacteria. A member of the *Phlebia* spp. was identified as a key OTU for DEC litters with a strong positive relationship to polyphenols. This is significant as *Phlebia* are often lignin degrading white rot fungi, which have been shown to have high polyphenol oxidase activity (Talbot *et al.*, 2015). While the symbiosis of bacterial and fungal decomposers has been remarked upon before in terrestrial systems (e.g. De Boer *et al.*, 2005), to our knowledge no one has explored their dynamics in lakes sediments before. These dynamics are important to understand as the phenolic compound levels in plant tissues is predicted to increase with rising atmospheric CO_2 levels (Tuchman *et al.*, 2002).

Bacterial communities

We observed a reduction in bacterial diversity with increasing concentrations of plant litter and hypothesize that this reflects a specialization of the community (Fig.2A). This trend was previously observed using additions of OM and is surmised to be the effect of environmental filtering, as the bacteria that dominate are adapted to thrive in the chemical environments created by different types of OM (e.g. Sato *et al.*, 2016). We saw evidence of environmental filtering in comparing the un-weighted UniFrac distances (which incorporate presence-absence data with phylogenetic distances; Fig. 1A) with the weighted UniFrac distances (which also incorporated relative abundances) (Fig. S1A), which revealed the selection of certain bacterial taxa across increasing polyphenol levels (pattern displayed in Fig. 5). Although the bacteria present were very similar between DEC and CON treatments, the chemical composition of the plant litter

selected for different dominant bacterial taxa. The spiked-sediments resulted in different dominant bacterial taxa in the DEC 40% OM treatments, which were dominated by Lactobacillales and Rhodospiralles versus the un-spiked treatments that were dominated by Bacillales (Fig. 5). Szkokan-Emilson *et al.*, (2017) found that the spiked DEC treatment had lower polyphenol levels then the un-spiked DEC treatment (Fig. 5), suggesting the spike of high OM sediments contained microbes that the un-spiked community did not possess, and which were better able to metabolize polyphenols above a certain threshold. Adapting to high polyphenol concentrations could be important to C cycling in lake sediments, as polyphenols are often regarded as inhibitors of decomposition and nutrient cycling (Hättenschwiler and Vitousek, 2000). The differences observed here between the un-spiked and spiked sediment communities to process polyphenols suggests that natural lake sediments range in their abilities to process fresh leaf litter and this could become important in the context of changing forests that surround lakes.

Fungal communities

The common paradigm that bacteria dominate anaerobic sediments and soils over fungi has led to fungi being often ignored or not reported in literature examining decomposition of OM in lake sediments. In aerobic conditions, plant litter is colonized by fungi that often have greater biomass than bacteria (e.g. Newell *et al.*, 1995; Findlay *et al.*, 2016). The aerobic activities of fungi as decomposers contributing to C cycling in aquatic systems has been observed and acknowledged (see Bärlocher, 2016), however little prior evidence suggested important roles in anaerobic degradation of organic inputs to sediment. Here our data show fungal communities maintain the dominant taxa that are defined by litter type (comprised of various plant pathogens,

yeasts, black yeasts and yeast like fungi, see Fig. 3), but have variable low abundant taxa in response to anaerobic conditions (many of which are unidentifiable at or below phylum level). With the presence of high polyphenols in the DEC treatment samples it is likely that bacterial decomposers were out-competed by fungal taxa better adapted to metabolizing polyphenols (i.e. *Phlebia* spp.). It is also likely the litters leached polyphenols with different anti-microbial properties that contributed to an environmental filtering effect, as different polyphenolic compounds can be inhibitory to different microbial groups (Daglia, 2012). The opposite case was likely true with the lower polyphenol conditions, where the fungi were unable to compete as well with bacteria.

Methanogen communities

Over the 150-day incubation we observed increasing amounts of CH_4 production from amended sediments and sequencing revealed a specific taxon responsible for carrying out methanogenesis. Overall the TYP treatment communities were dominated by Methanosarcinaceae (OTU 2, grouping with *Methanosarcina barkeri* in Fig. 4), and Methanobacteriaceae (OTU 1, further identified as *Methanobacterium sp.* swan-1), however increasing CH_4 production corresponded with the increase in Methanosarcinaceae in the 10% and 20% OM TYP treatments, and notably members of this family have relatively wide substrate utilization capabilities (De Vrieze *et al.*, 2012; Liu and Whitman, 2008). In contrast the 40% OM treatment (both un-spiked and spiked) had lower *mcr*A copies and was dominated by Methanobacteriaceae, typically restricted to CO_2 reduction methanogenic pathways (De Vrieze *et al.*, 2012; Liu and Whitman, 2008). This pattern could have been due to a lag in available metabolites for methanogens, a pattern supported by the temporal lag seen in CH_4 production

with increasing TYP treatment concentrations over the 150-day incubation (Fig. S5). The delay in the onset of methanogenesis could have been be due to competition by other microbial guilds (e.g. sulfate reducers also capable of H^+ or acetate utilization) dominating when larger amounts of fresh litter were present. A similar temporal pattern was previously reported in an experiment examining the degradation of switchgrass in a rumen environment, where 13% of the biomass was degraded in the first 30 minutes, followed by 3.5 hour period of reduced biomass degradation where methanogens increased in cell number 3-fold (Piao *et al.*, 2014). Although during our 150-day incubation methanogenesis was inhibited by polyphenols, longer term studies would be useful to examine how polyphenolic compounds are further processed and if methanogenesis rates subsequently increase.

Conclusions

Our study demonstrates how lake sediment microbial communities are intertwined in the processing and fate of terrestrial organic inputs. We observed changes in the composition, diversity and abundance of bacteria, fungi and methanogens in our sediments amended with different plant litters. The bacterial orders that dominated the different litters changed with the different polyphenol content in the different litters, showing a specialization of the community to specific litter types. In a broader ecological context, our study provides evidence for the mechanism by which sediment CH_4 production is modulated by plant litter compositions through changing methanogen community compositions. We also showed that fungi can be important decomposers in anaerobic lake sediments with high polyphenolic content plant litter, with corresponding dominance of a specialized polyphenol decomposer, *Phlebia* spp. Therefore, the data presented in this study provides valuable baseline knowledge to further explore how

microbial communities will deal with changing C sources.

Acknowledgements

We acknowledge the support of staff and colleagues at the Living with Lakes Centre, Laurentian University, in Sudbury Canada. Funding was provided by NERC Standard Grant NE/L006561/1.

Author Contributions

KY, ESE, MC, AJT, NB, and NM conceived the study. KY, ESE, and MC collected the data, and KY analyzed the data and wrote the manuscript with input from all authors.

References:

Angel R, Claus P, Conrad R. (2011). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. *ISME J* **6**: 847–862.

Bärlocher F. (2016). Aquatic fungal ecology. *Fungal Ecol* **19**: 1–4.

Bastviken D, Cole JJ, Pace ML, Van de Bogert MC. (2008). Fates of methane from different lake habitats: Connecting whole-lake budgets and CH_4 emissions. *J Geophys Res* **113**: G02024.

Bastviken D, Tranvik LJ, Downing J, Crill J a, M P, Enrich-prast A. (2011). Freshwater Methane Emissions Offset the Continental Carbon Sink. *Science* **331**: 50.

De Boer W, Folman LB, Summerbell RC, Boddy L. (2005). Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiol Rev* **29**: 795–811.

Bokulich NA, Mills DA. (2013). Improved Selection of Internal Transcribed Spacer-Specific Primers Enables Quantitative , Ultra-High-Throughput Profiling of Fungal Communities. *Appl Environ Microbiol* **79**: 2519–2526.

Bouzat JL, Hoostal MJ, Looft T. (2013). Spatial patterns of bacterial community composition within Lake Erie sediments. *J Great Lakes Res* **39**: 344–351.

Brad T, Braster M, Van Breukelen BM, Van Straalen NM, Röling WFM. (2008). Eukaryotic diversity in an anaerobic aquifer polluted with landfill leachate. *Appl Environ Microbiol* **74**: 3959–3968.

De Caceres M, Legendre P. (2009). Associations between species and groups of sites: indices and statistical inference. *Ecology* **90**: 3566–3574.

Chen N, Yang J-S, Qu J-H, Li H-F, Liu W-J, Li B-Z, *et al.* (2015). Sediment prokaryote communities in different sites of eutrophic Lake Taihu and their interactions with environmental factors. *World J Microbiol Biotechnol* **31**: 883–896.

Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. (2016). GenBank. *Nucleic Acids Res* **44**: D67–D72.

Daglia M. (2012). Polyphenols as antimicrobial agents. *Curr Opin Biotechnol* **23**: 174–181.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, *et al.* (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* **72**: 5069–5072.

Downing JA, Prairie YT, Cole JJ, Duarte CM, Tranvik LJ, Striegl RG, *et al.* (2006). The global abundance and size distribution of lakes , ponds , and impoundments. *Limnol Oceanogr* **51**: 2388–2397.

Edgar RC. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5**: 1–19.

Field JA, Leyendekers MJH, Sierra Alvarez R, Lettinga G, Habets LHA. (1988). Methanogenic toxicity of bark tannins and the anaerobic biodegradability of water soluble bark matter.

Water Sci Technol **20**: 219–240.

Fierer N, Jackson JA, Vilgalys R, Jackson RB. (2005). Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays. *Appl Environ Microbiol* **71**: 4117–4120.

Findlay S, Howe K, Austin HKAY. (2016). Comparison of Detritus Dynamics in Two Tidal Freshwater Wetlands. *Ecology* **71**: 288–295.

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, *et al.* (2005). Global consequences of land use. *Science* **309**: 570–574.

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey DW, *et al.* (2007). Changes in Atmospheric Constituents and in Radiative Forcing. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, et al. (eds). Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA. https://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter2.pdf.

Gangneux C, Akpa-Vinceslas M, Sauvage H, Desaire S, Houot S, Laval K. (2011). Fungal, bacterial and plant dsDNA contributions to soil total DNA extracted from silty soils under different farming practices: Relationships with chloroform-labile carbon. *Soil Biol Biochem* **43**: 431–437.

Hättenschwiler S, Vitousek PM. (2000). The role of polyphenols in terrestrial ecoystems nutrient cycling. *Tree* **15**: 238–243.

Heyer C den, Kalff J. (1998). Organic matter mineralization rates in sediments: A within- and among-lake study. *Limnol Oceanogr* **43**: 695–705.

Hofmann H, Federwisch L, Peeters F. (2010). Wave-induced release of methane : Littoral zones as a source of methane in lakes. *Limnol Oceanogr* **55**: 1990–2000.

Hoostal MJ, Bouzat JL. (2008). The modulating role of dissolved organic matter on spatial patterns of microbial metabolism in Lake Erie sediments. *Microb Ecol* **55**: 358–368.

Koljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, *et al.* (2013). Towards a unified paradigm for sequence-based identification of fungi. *Mol Ecol* **22**: 5271–5277.

Kritzberg ES, Cole JJ, Pace ML, Granéli W, Darren L, Kritzberg ES, *et al.* (2004). Autochthonous versus allochthonous carbon sources of bacteria : Results from whole-lake 13C addition experiments. *Limnol Oceanogr* **49**: 588–596.

Kumar S, Stecher G, Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* **33**: 1870–1874.

Lecerf A, Risnoveanu G, Popescu C, Gessner MO, Chauvet E. (2007). Decomposition of diverse litter mixtures in streams. *Ecology* **88**: 219–227.

Liu Y, Whitman WB. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea.N.Y Acad. Sci. **1125**:171-189.

Logue JB, Stedmon C a, Kellerman AM, Nielsen NJ, Andersson AF, Laudon H, *et al.* (2015). Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *ISME J* 1–13.

Logue JB, Stedmon CA, Kellerman AM, Nielsen NJ, Andersson AF, Laudon H, *et al.* (2016). Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *ISME J* **10**: 533–545.

Love MI, Huber W, Anders S. (2014). Moderated estimation of fold change and dispersion for RNA-seq

data with DESeq2. *Genome Biol* **15:550**.

Maie N, Pisani O, Jaffe R. (2008). Mangrove tannins in aquatic ecosystems: Their fate and possible influence on dissolved organic carbon and nitrogen cycling. *Limnol Oceanogr* **53**: 160–171.

Maie N, Scully NM, Pisani O, Jaffé R. (2007). Composition of a protein-like fluorophore of dissolved organic matter in coastal wetland and estuarine ecosystems. *Water Res* **41**: 563–570.

Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. (2012). PANDAseq : PAired-eND Assembler for Illumina sequences. *BMC Bioinformatics* **13**: 1–7.

Mcinerney MJ, Sieber JR, Gunsalus RP. (2009). Syntrophy in anaerobic global carbon cycles. *Curr Opionion Biotechnol* 623–632.

McMurdie PJ, Holmes S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One* **8**: e61217

Millar CI, Stephenson NL, Stephens SL. (2007). Climate Change and Forts of the Future: Managing in the Face of Uncertainty. *Ecol Appl* **17**: 2145–2151.

Newell SY, Moran MA, Wicks R, Hodson RE. (1995). Productivities of microbial decomposers during early stages of decomposition of leaves of a freshwater sedge. *Freshw Biol* **34**: 135–148.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, *et al.* (2017). vegan: Community Ecology Package. https://cran.r-project.org/package=vegan.

Piao H, Lachman M, Malfatti S, Sczyrba A, Knierim B, Auer M, *et al.* (2014). Temporal dynamics of fibrolytic and rnethanogenic rumen microorganisms during in situ incubation of switchgrass determined by 16S rRNA gene profiling. *Front Microbiol* **5**:81-91.

R Core Team. (2016). R: A language and environment for statistical computing. *R Found Stat*

Comput. https://www.r-project.org/.

Sato Y, Hori T, Navarro RR, Habe H, Yanagishita H, Ogata A. (2016). Fine-scale monitoring of shifts in microbial community composition after high organic loading in a pilot-scale membrane bioreactor. *J Biosci Bioeng* **121**: 550–556.

Schindler MH, Gessner MO. (2009). Functional leaf traits and biodiversity effects on litter decomposition in a stream. *Ecology* **90**: 1641–1649.

Stamatakis A. (2014). RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics*. **30**:1312-1313.

Stamatakis A, Zhang J, Kobert K. (2014). Genome analysis PEAR : a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30**: 614–620.

Swan CM, Gluth MA, Horne CL. (2009). Leaf litter species evenness influences nonadditive breakdown in a headwater stream. *Ecology* **90**: 1650–1658.

Szkokan-Emilson EJ, Carson MA, Yakimovich KM, Gunn JM, Mykytczuk NCS, Basiliko N, *et al.* (2017). Climate-driven shifts in sediment chemistry enhance methane emissions from northern lakes. *Nat Clim Chang* **In Review**.

Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M. (2014). Development of a Prokaryotic Universal Primer for Simultaneous Analysis of Bacteria and Archaea Using Next-Generation Sequencing. *PLoS One* **9**: e105592.

Talbot JM, Martin F, Kohler A, Henrissat B, Peay KG. (2015). Functional guild classification predicts the enzymatic role of fungi in litter and soil biogeochemistry. *SOIL Biol Biochem* **88**: 441–456.

Tranvik LJ, Downing J a., Cotner JB, Loiselle S a., Striegl RG, Ballatore TJ, *et al.* (2009). Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol Oceanogr* **54**: 2298–

2314.

Tuchman NC, Wetzel RG, Rier ST, Wahtera KA, Teeri JA. (2002). Elevated atmospheric CO2 lowers leaf litter nutritional quality for stream ecosystem food webs. *Glob Chang Biol* **8**: 163–170.

Vavrek MJ. (2011). fossil: palaeoecological and palaeogeographical analysis tools. *Palaeontol Electron* **14**: 1T.

De Vrieze J, Hennebel T, Boon N, Verstraete W. (2012). Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. *Bioresour Technol* **112**: 1–9.

Wardle DA, Bonner KI, Nicholson KS. (1997). Biodiversity and plant litter: Experimental evidence which does not support the view that enhanced species richness improves ecosystem function. *OIKOS* **79**: 247–258.

Warnes MGR, Bolker B, Bonebakker L. (2016). Package 'gplots'. *Var R Program Tools Plotting Data*.

West WE, Coloso JJ, Jones SE. (2012). Effects of algal and terrestrial carbon on methane production rates and methanogen community structure in a temperate lake sediment. *Freshw Biol* **57**: 949–955.

West WE, McCarthy SM, Jones SE. (2015). Phytoplankton lipid content influences freshwater lake methanogenesis. *Freshw Biol*. **60**:2261-2269.

Wetzel RG. (1992). Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic matter in freshwater ecosystems. *Hydrobiologia* **229**: 181–198.

Wurzbacher CM, Barlocher F, Grossart HP. (2010). Fungi in lake ecosystems. *Aquat Microb Ecol* **59**: 125–149.

Yamashita Y, Jaffé R, Maie N, Tanoue E. (2008). Assessing the dynamics of dissolved organic

matter (DOM) in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC). *Limnol Oceanogr* **53**: 1900–1908.

Ye R, Keller JK, Jin Q, Bohannan BJM, Bridgham SD. (2016). Peatland types influence the inhibitory effects of a humic substance analog on methane production. *Geoderma* **265**: 131–140.

Tables and figures:

Table 2.1: Quantitative PCR of Bacteria (16S) and Fungi (18S) in sediments and the starting litter OM used to amend the treated sediments. Values are the mean copy number of spiked and un-spiked samples with standard error in brackets calculated from standard curves of pure cultures.

Treatment	N	Fungal Copies	Bacterial Copies	Ratio of Fungi to Bacteria	Relative polyphenol concentration
CON Sediment	5	36,336 (12,908)	277,394 (49,311)	0.13 (0.029)	21 (0.015)
DEC Sediment	6	95,085 (13,393)	68,700 (18,958)	1.9 (0.518)	43 (0.075)
TYP Sediment	6	13,364 (2,585)	962,674 (112,875)	0.013 (0.001)	6.6 (0.016)
CON Litter	1	5,318,182	36,363	146.3	37
DEC Litter	1	1,896,899	75,271	25.2	34
TYP Litter	1	4,240,310	257,286	16.5	12

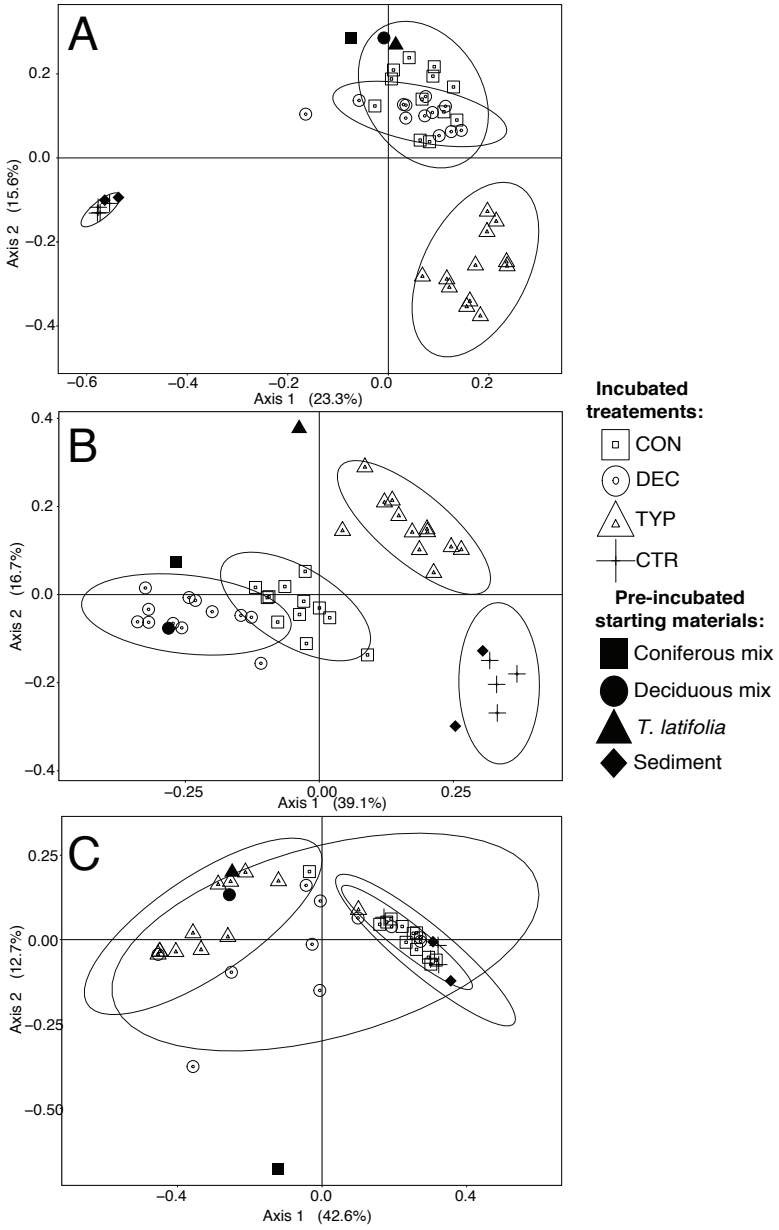


Figure 2.1: PCoAs of un-weighted UniFrac distances using relative abundances for the respective microbial communities; bacteria (A; 5565 OTUs), fungi (B; subset of 128 OTUs), and methanogens (C; 43 OTUs), across all treatment concentration. Ellipses represent 95% confidence intervals which were calculated for each incubated treatment. The proportion of variation explained by each axis is given in parentheses.

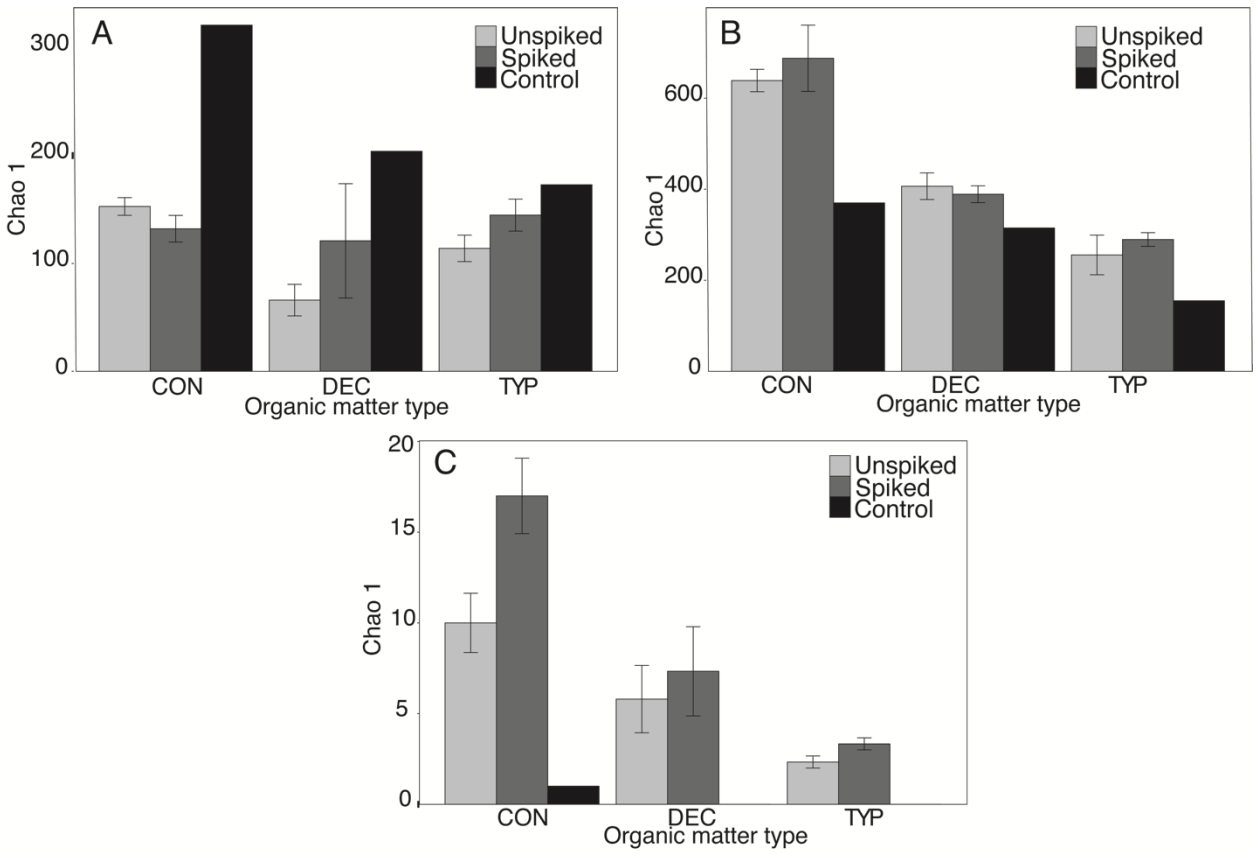


Figure 2.2: Mean Chao 1 diversity values (±SE) of bacterial (A), fungal (B) and methanogen (C) communities for each plant litter type. Diversity was calculated as Chao 1 values averaged for each plant litter type added to either the un-spiked or spiked amended sediments and pre-incubattion plant litter controls.

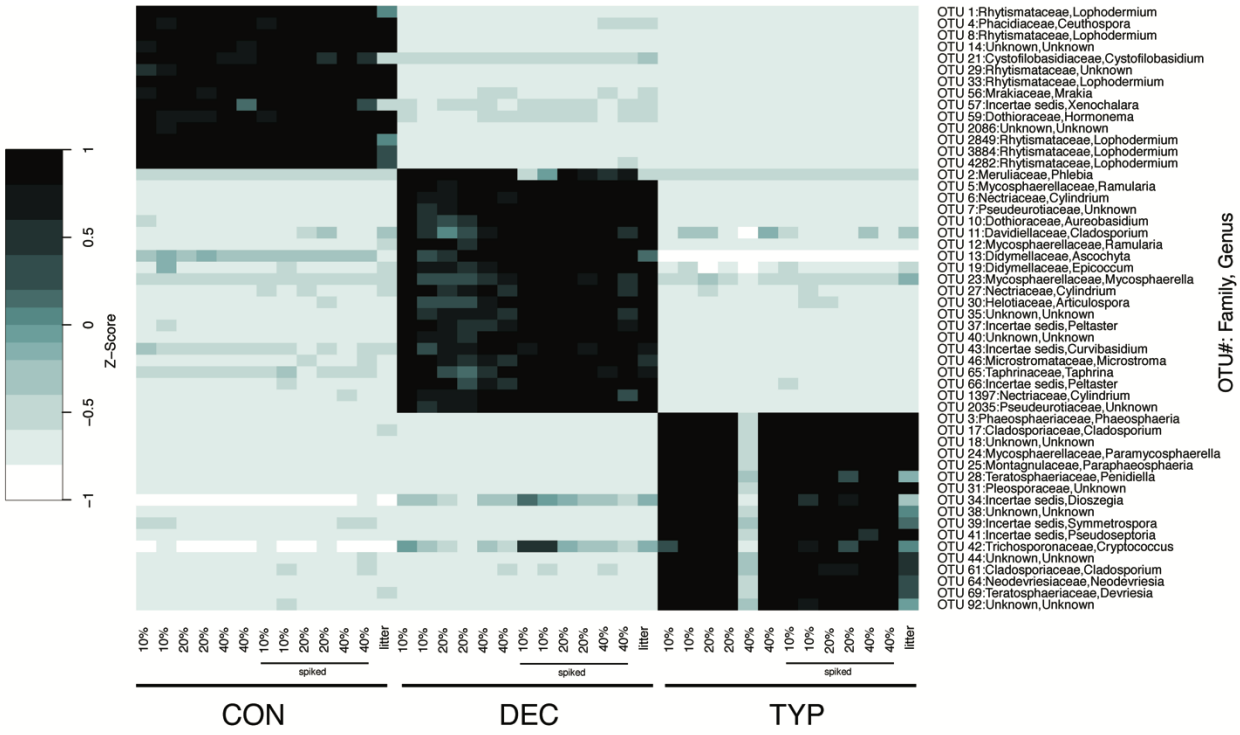


Figure 2.3: Heat map of indicator fungal OTUs for replicate DNA extractions by organic matter type and their respective starting litter material. OTU number and assigned taxonomic information are identified on the right y-axis.

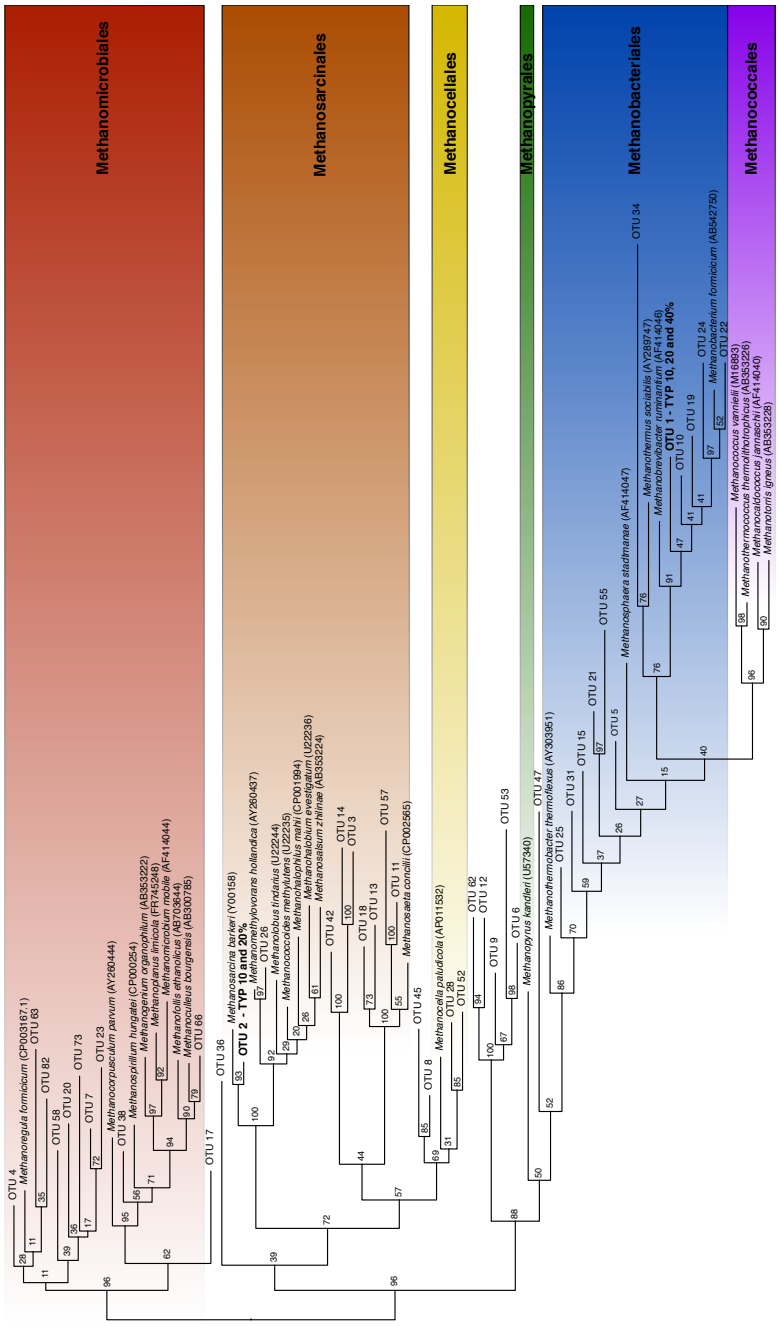


Figure 2.4: Maximum-likelihood phylogenetic tree of the *mcr*A gene containing the 43 OTUs found in all treatments. Node values are from 1000 bootstrap replicates and branches were arranged and highlighted by methanogen orders. Reference sequences from described species were selected to represent the orders of methanogens and were obtained from GenBank and their tip labels contain their accession number.

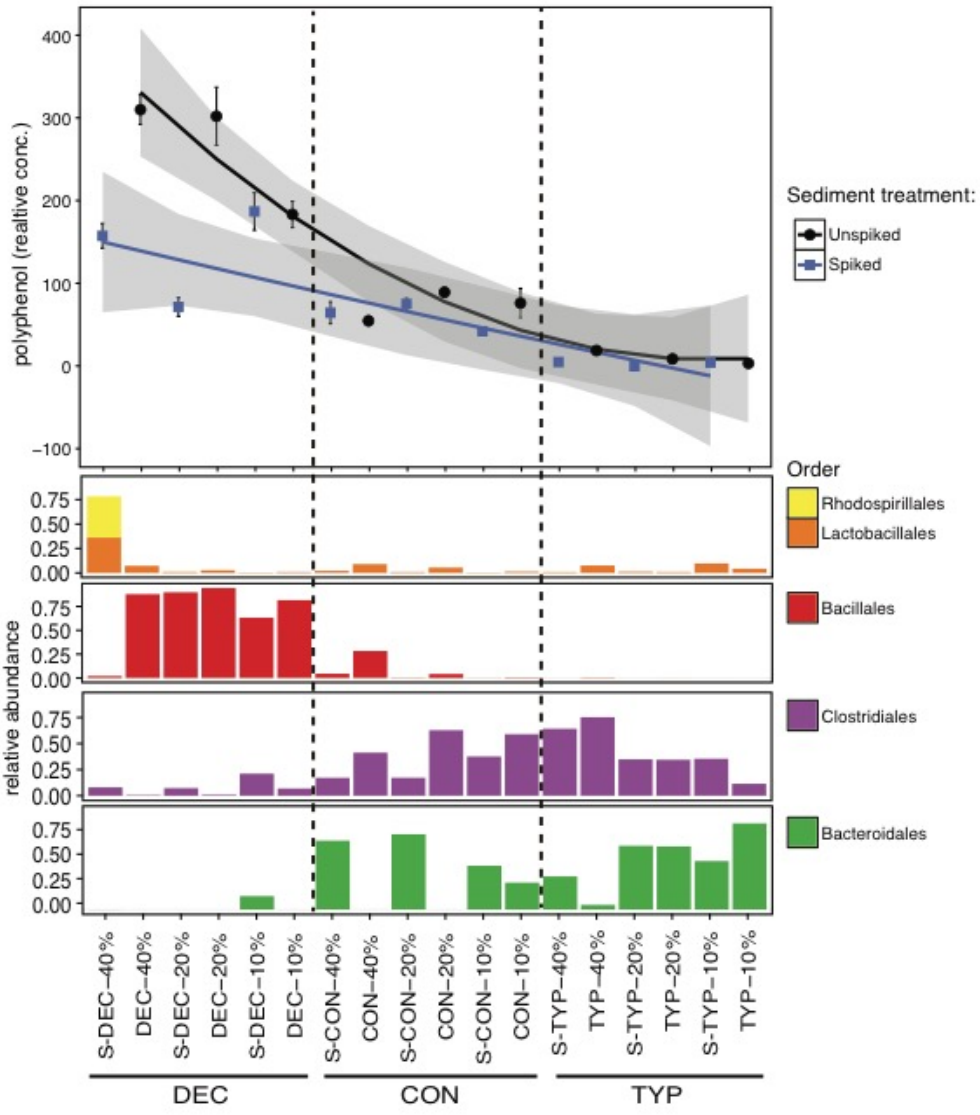
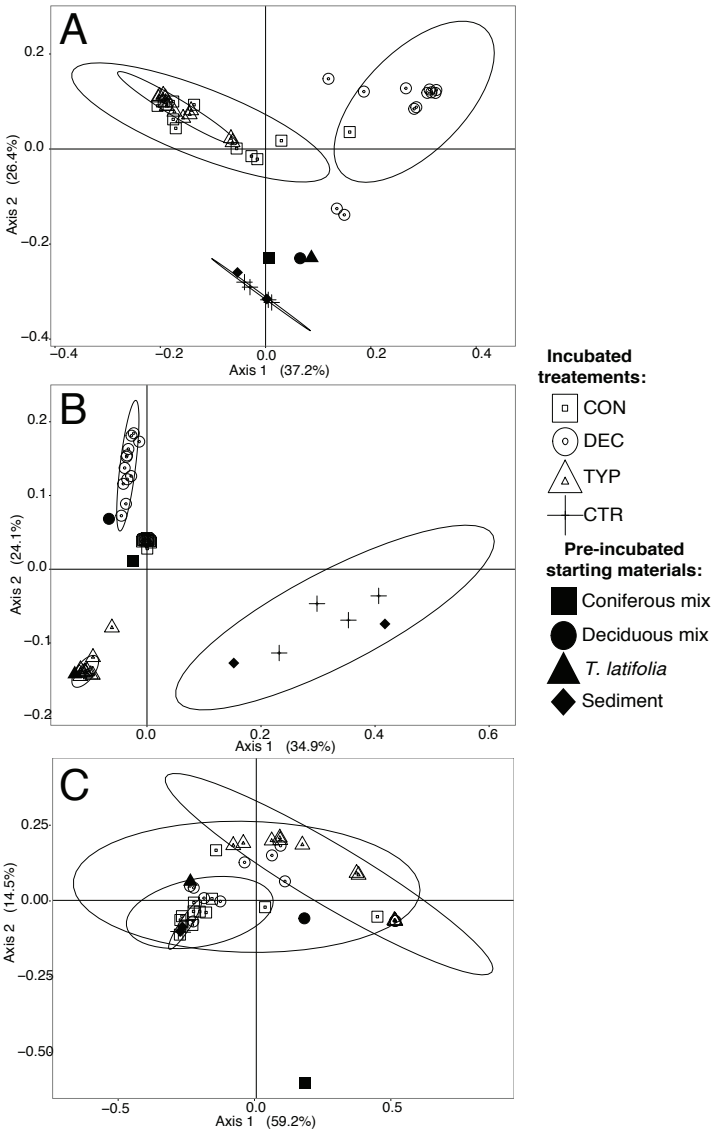
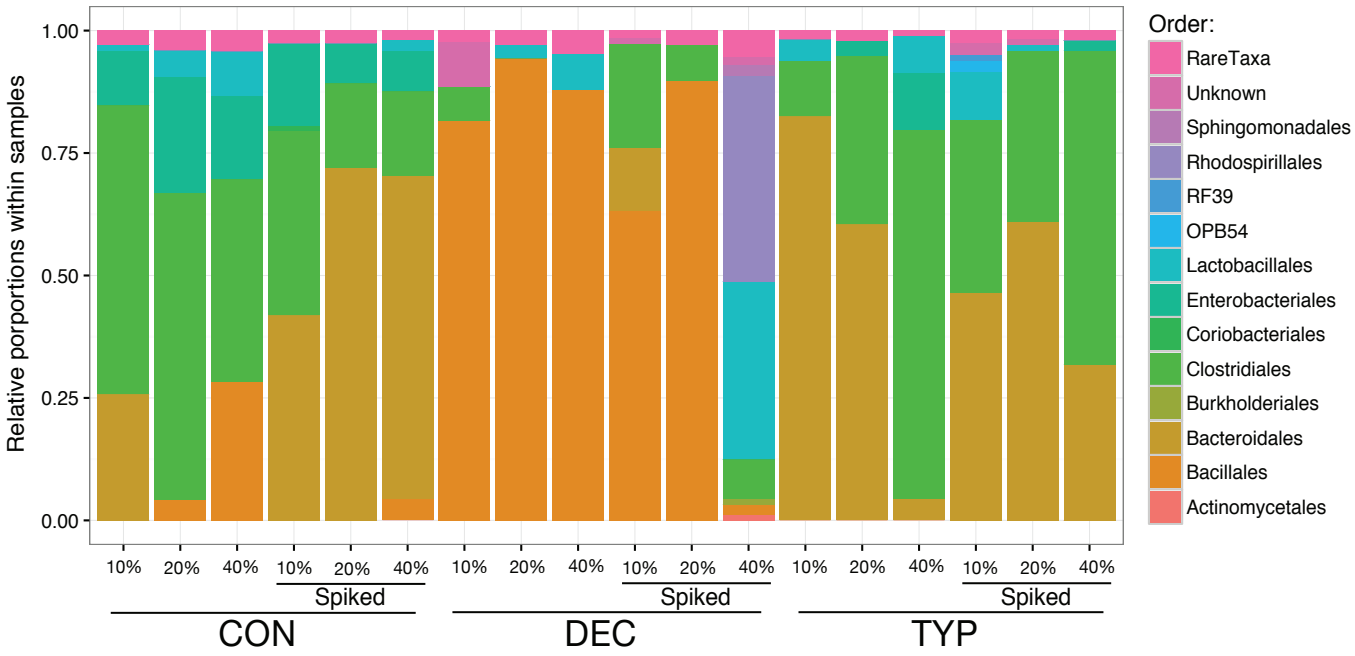


Figure 2.5: Relative polyphenol concentrations across leaf litter types correspond with shifts in dominant bacterial orders. Polyphenol concentrations were standardized to relative concentrations of total dissolved organic carbon. Methanogen spiked samples are indicated on the x-axis labels with S. The x-axis was ordered to create a pseudo-concentration gradient of polyphenols across three plant litters with dashed lines denoting different litter types. Polyphenol levels were averaged for each treatment and bars represent standard errors with the shaded area around each trend line representing the 95% confidence interval. Relative abundances from sequencing duplicates were averaged for every OTU to represent each treatment and were then summed by Order.

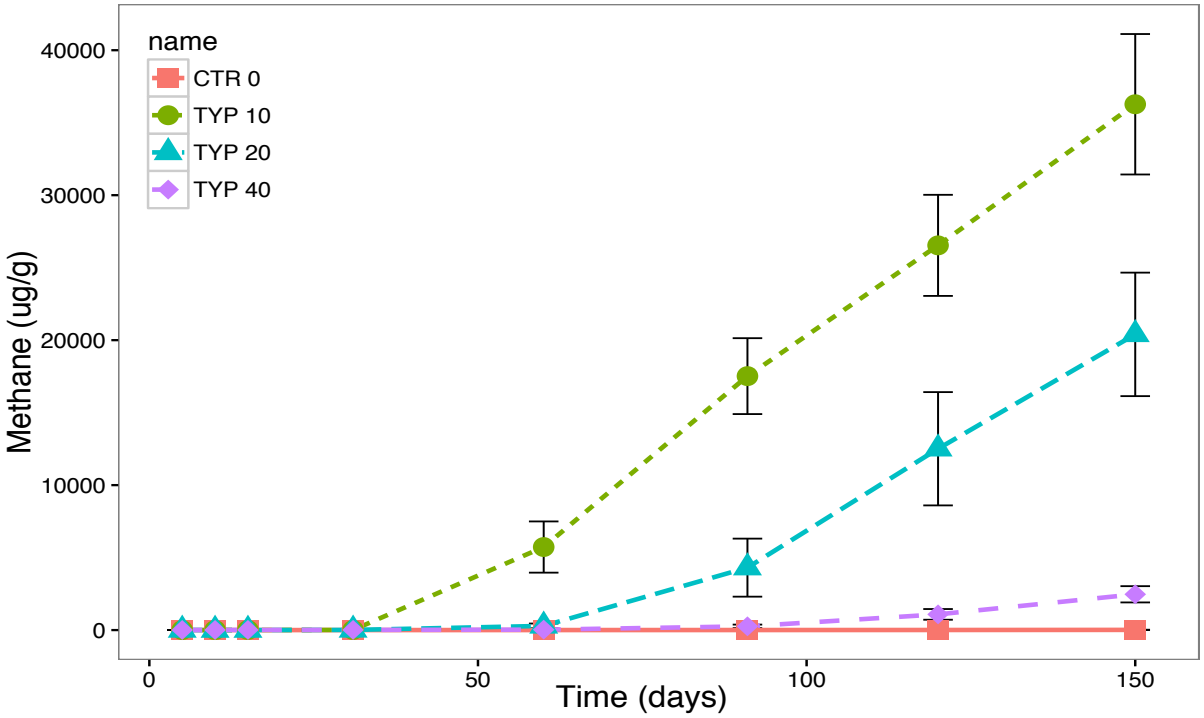
Appendix 1: Chapter 2 supplementary figures



Supplementary Figure 1: PCoAs of weighted UniFrac distances using relative abundances for the respective microbial communities; bacteria (A; 5565 OTUs), fungi (B; subset of 128 OTUs), and methanogens (C; 43 OTUs), across all treatment concentration. Ellipses represent 95% confidence intervals which were calculated for each incubated treatment. The proportion of variation explained by each axis is given in parentheses.



Supplementary Figure 2: Relative abundance of each litter sample and concentration for bacterial orders. Values are the average of replicate sequencing runs. All Orders that were present as <1% relative abundance were grouped as Rare Taxa.



Supplementary Figure 3: Cumulative production of CH_4 over time for the TYP treatment, displayed by percent organic matter. Points are means with ±SE for µg methane in g per dry weight of sediment.

Chapter 3: Photoexposure accelerates terrestrial plant litter decomposition rates and subsequently methanogenesis in lake sediments

Kurt M. Yakimovich[1,3], Chloe Orland[4], Erik J. Szkokan-Emilson[4], Andrew J. Tanentzap[4], Nathan Basiliko[1,3] and Nadia C.S. Mykytczuk[1,2,3]

[1] Vale Living with Lakes Centre, Laurentian University, Sudbury ON, Canada
[2] School of the Environment, Laurentian University, Sudbury ON, Canada
[3] Department of Biology, Laurentian University, Sudbury ON, Canada
[4] Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom

Abstract:

Lake sediments harbor microbial communities responsible for the degradation of organic matter and its eventual mineralization to CO_2 and CH_4. Key to this are the methanogen communities that are responsible for one of the terminal steps of anaerobic decomposition. Here we deployed mesocosms of constructed sediments into three lakes in Sudbury, ON, with controlled amounts of deciduous and coniferous tree litters. Pore water and sediment samples were collected once a month from August – October, and were used to assess the difference in methanogen community composition and organic matter decomposition over time and in the different lakes. We found that the mesocosms from all lakes were largely dominated by the same two methanogens that are members of the order Methanobacteriales. The dynamics of the two dominant methanogens were affected by pH changes resulting from leaf litter decomposition, however no large differences existed between the decomposition of the deciduous or coniferous litters. Additionally, a specialist species, *Methanomassiliicoccus luminyensis* B10, was found to increase in abundance over time and appeared responsible for increased methanogenesis. Overall decomposition and methane and carbon dioxide production was enhanced in our least turbid study lake relative to the other two lakes, where the mesocosms had the most photoexposure. Our study demonstrates how differing physicochemical factors affect the activity and composition of lake sediment methanogen communities.

Introduction:

Methanogens in lake sediments are involved in the terminal decomposition of organic matter (OM) by mineralizing it to CH_4. As a gas CH_4 can either get oxidized in lake waters and enter microbial biomass or be emitted to the atmosphere. These potential emissions are important

as CH_4 is a greenhouse gas ~25X more potent than CO_2 (Forster *et al.*, 2007). Therefore, understanding the microbial communities driving methanogenesis is key to understanding the role of lake sediments in the global climate system and biospeheric feedbacks to environmental changes. Especially important for budgeting CH_4 emissions in lakes are littoral (shallow) lake sediments, where evasion of CH_4 to the atmosphere can be assisted by various mechanisms such as natural and anthropogenic wave action, ebullition, or plant-mediated diffusion, all which can reduce residence time of CH_4 in the oxic water column (Hofmann *et al.*, 2010).

Some previous research focusing on the diversity and community structure of methanogens in lake sediments. For example, in the profundal sediments of Lake Pavin (France), co-dominance by the methanogen orders Methanomicrobiales and Methanosarcinales was found (Biderre-Petit *et al.*, 2011). It has been previously hypothesized that the contrasting pathways of CO_2 reduction and acetotrophy between members of these two Orders collectively represent a syntrophy leading to the complete removal of H_2 and acetate during terminal anaerobic OM degradation (Schwarz *et al.*, 2007). In a study of vertical sediment profiles in two lakes on the Yunnan Plateau (China), it was found that Methanomicrobiales was the dominant methanogen group in all sediment depths over Methanosarcinales, and with Methanobacteriales making up high proportions in surface layer sediments (Yang *et al.*, 2017). However, this literature doesn't link methanogen taxa to methanogenesis rates, therefore detectable diversity via gene surveys doesn't necessarily reveal methanogen community functioning. Whereas in a study of cow rumens directly linked the increase in abundance of a specialist methanogen clade to increases in methanogenesis (Danielsson *et al.*, 2017), which reveals that higher methane production can be linked to single specialist taxa. These creates the question on the differences in functionality of communities that are co-dominated by methanogens participating in symbiosis versus those that

are dominated by a specialist methanogen that can increase in abundance and activity. Additionally, how do influxes of OM sources effect the dynamics between specialist methanogens that have a narrow niche versus relatively more generalist taxa?

The diversity of methanogens in lake sediments has been previously linked to physicochemical factors including total organic carbon (TOC) and other nutrients (e.g. Yang *et al.*, 2017). This raises questions of how specific sources of organic matter influence lake sediment methanogen communities. OM enters lakes either from external and often terrestrial sources (allocthonous) or from internal sources such as aquatic vegetation or phytoplakton (autochthonous). Autochothonous sources of OM have experimentally been found to be rapidly utilized by microbes, leaving a majority of OM that resides in sediments to be terrestrial in origin (Kritzberg *et al.*, 2004; West *et al.*, 2012). However, little is known on how differeing quanitites and qualities of terrestrial OM affects sediment methanogens, and how community dynamics change temporally. In light of changing plant communities in catchments feeding lakes under land use and climate change scernarios, it is important to investigate how different terrestrial plant litter inputs ot lakes influence methanogen community composition and activity.

Here we used experimentally constructed lake sediment mesocosms to explore methanogen community establishment under different litter input scenarios. We used both a deciduous and coniferous tree litter mix making sediments of varying OM quality and quantity which were replicated across three lakes to assess the impact of different lake physicochemical parameters. We sampled the mesocosms once every month over the first three months of decomposition which allowed us to explore: 1) How does the ratio of deciduous and coniferous litter effect the methanogen community composition? 2) Are the dominant methanogens in the same sediment amendments the same across lakes? 3) Is there a specific dominant methanogen

taxon linked to rapid methanogenesis? 4) How does the methanogen community change over the first three months of fresh litter decomposition? These questions allow for a focused analysis of how different tree leaf litters influence sediment methanogen communities, and ultimately how changes in plant communities in the uplands might lead to climate change feedbacks in lakes.

Methods:

Methodologies for this study were first published in Tanentzap *et al.* (2017), which demonstrate that our experimental methodology is capable of replicating conditions observed in natural lake sediments, allowing for realistic observations to be made in our present study. In brief, for this study we constructed sediment mesocosms and installed them into three lakes with differing chemical properties in Sudbury, ON: Ramsey Lake (46°28'42.1"N 80°56'30.4"W), Swan Lake (46°21'57.3"N 81°03'54.5"W) and Laurentian Lake (46°27’30” N, 80°56’0” W). Laurentian lake is oligotrophic and 1.35 km^2 with 16.68% of its catchment area consisting of wetlands and is within the Lake Laurentian Conservation Area, with high relative DOC and low visibility and nutrients. Swan Lake is also oligotrophic and 0.058 km^2 in size, surrounded by forested areas with relatively low DOC, low nutrients and high visibility. Ramsey Lake is mesotrophic 7.96 km^2, surrounded by urban development of the City of Sudbury with low dissolved organic content (DOC) with high relative nutrient levels and visibility. These three lakes were chosen to provide variation in physicochemical conditions and asses how those potentially related to methanogen communities and activities in along with the organic matter loading experiment. All mesocosms were sampled on one date in each of the months August,

September and October. Our sampling scheme allowed for a temporal examination of the mesocosms and captured a seasonal change from summer to fall.

Mesocosm construction

Deciduous (primarily *Acer rubrum*, *Betula papyrifera*, *Populus tremuloides*, *Quercus* spp.) and coniferous (*Pinus* spp.) tree leaf litter were selected for this experiment as they are typical of terrestrial vegetation surrounding region lakes. The litter was first mulched to reduce its size, and then sifted through 1 cm^2 and 1 mm^2 mesh to collect two size fractions to better simulate particle size observed in natural sediments (Tanentzap *et al.*, 2017). The different OM types were then mixed together in precise proportions to prepare treatments as shown in Table 1. A mix of 7:3 by weight of the large size fraction (>1mm) to smaller size fraction (<1mm) was prepared for both the broadleaf and coniferous OM material. An underlying inorganic mix was 30:50:20 mix by weight of gravel, sand and clay respectively. The treatments of OM:inorganic matter mix was deposited into plastic bins (surface area: 0.19 m^2, depth: 0.13 m, to form mesocosms), on top of 4.5 kg of ballast gravel, adjusting the added weight of each treatment to a similar volume for all bins (i.e. to ensure the OM came above the water sampling port that is described below). Once filled with artificial sediment, the mesocosms were covered in a fine 1mm^2 mesh to keep the tree litter contained during initial installation and decomposition while densities were low—and the mesh remained on for the duration of this study. Each mesocosm was fitted with a pore water sampler made from a 3mL syringe that had a slit cut into the bottom, wrapped in a fine mesh material to filter large particles out of water and inserted horizontally in through a hole drilled 1cm below the sediment surface. A plastic tube was fitted to the tip of the syringe extending up to a float on the surface of the water, to allow for sampling of pore water

from each mesocosm (see Tanentzap *et al.* (2017) for depiction). The mesocosm installations in each lake were equipped with 12 HOBO temperature/light data loggers, set to take hourly readings (Onset Computer Corporation, USA). The mesocosms were installed in littoral zones at ~ 0.5m water depth in each lake.

Pore water measurements

Our setup allowed water samples to be taken from each mesocosm and sampled for pH, pore water CO_2 and CH_4 levels, total dissolved organic carbon (DOC) as well as analyses of the chemical properties of the dissolved organic matter (DOM). During field sampling, pH was measured immediately, then samples were filtered through a 0.4 μm pore size silica filters into 25 mL glass vials and acidified with 125 μL of 4M HCl as a preservative for future analysis of DOC and DOM. DOM chemical properties were assessed via fluorescence EEMs (excitation-emissions matrices) measured on an Agilent Cary Eclipse fluorescence spectrophotometer in ratio (S/R) mode with a 1-cm path-length cuvette. EEMs were created by excitation and emission intensities (EX: 250 to 450nm in 5nm steps, EM: 300 to 600nm in 2nm steps) corrected for inner-filter effects using absorbance measured with an Agilent Cary 60 UV-VIS. DOC was measured using a Shimadzu TOC-5000A in FPOC mode. Final DOM metrics used in our analysis included the humification index (mHIX), with higher values indication greater humified DOM present, and fluorescent index (FI) which is used to differentiate between terrestrially and microbially derived C (Hansen *et al.*, 2016).

For analysis of CH_4 and CO_2, another syringe was filled to 43 mL with pore water, then acidified with 2 mL of 0.5M HCl in the field. 15 mL of atmospheric air was pulled in, the syringes were shaken for 2 minutes, and then left to sit for 30 seconds to equilibrate. 10 mL of

the headspace was drawn into a syringe, and analyzed on a SRI 8610C-0040 Greenhouse Gas model gas chromatograph (Torrance, CA) fitted with a 0.5 ml sample loop and a column temperature of 105°C, and detected gases detected with a flame ionization detector and methanizer for CO_2. All CO_2 and CH_4 measurements were performed within 24 hours of field sampling and final pore water concentrations were calculated using the methods of Aberg and Wallin (2014) by subtracting ambient air additions, applying the Bunsen solubility coefficient and ideal gas law while accounting for pH and water temperature.

Methanogen community analysis

To identify/characterize the methanogen communities present in the mesocosms, sediment samples were collected by extracting surface sediments (~ top 5 cm) with a sterilized scoop (sterilized using 70% ethanol) through an 8cm slit in the mesh covering the mesocosms. The scoop was filled such that lake water was not included in samples as they were deposited into individual sterile sample bags (Whirl-Pak®). Samples were frozen at -20°C within a few hours after collection and remained frozen until being freeze-dried at -40°C. DNA was extracted from lyophilized sediments using the MoBio PowerSoil kit (MoBio, Carlsbad, CA, USA). Sequencing libraries were prepared using a dual indexing strategy with unique 8-bases indeces added to the primers to allow multiplexing of pooled libraries. Samples were amplified using 2µL of the forward and reverse primers: mlasF (5'-GGT GGT GTM GGD TTC ACM CAR TA-3') and mcrA-rev (5'-CGT TCA TBG CGT AGT TVG GRT AGT-3') to target the methanogen functional gene *mcr*A (Angel *et al.*, 2011). This was done using 10µL of Qiagen Multiplex PCR Master Mix (Qiagen, Valencia, CA, USA), 4µL of sterile double-distilled water and 2µL (10 ng/µL) of DNA extract in a total volume of 20µL. The following cycling conditions: initial

denaturation of 15 min at 95˚C, 35 cycles at 94˚C for 30 s, 55˚C for 45 s, 72˚C for 30 s, and final elongation of 10 min at 72˚C. Index primers were then added through a second amplification step, using reactions containing 2µL of both the forward and reverse indexing primers (Fi5XX and Ri7XX respectively, 1µM each) and 10µL of Qiagen Multiplex PCR Master Mix (Qiagen), and 8µL of template DNA, totaling a 22µL reaction volume. The following reaction conditions were used: initial denaturation of 15 min at 95˚C, 10 cycles at 98˚C for 10 s, 65˚C for 30 s, 72˚C for 30 s, and final elongation of 5 min at 72˚C. Resulting amplicons were quantified pooled in groups of 8 in equimolar quantities (150 ng). Final libraries were purified using an Agencourt AMPure XP beads kit (Beckman Coulter Genomics, Indianapolis, IN). Amplicons were quantified via qPCR reactions with 6µL of KAPA SYBR FAST mix and primers (KAPA Biosystems, Wilmington, MA, USA) and 2µL water, with the following reaction conditions: 95˚C for 5 min, 35 cycles at 95˚C for 30s and 60˚C for 45 sec. Amplicon size was checked via an Agilent 4200 TapeStation (Agilent, Santa Clara, CA) and pooled into a single sample in equimolar concentrations. The final library concentration was found using Quant-it PicoGreen dsDNA Assay kit (Invitrogen, Eugene, OR, USA). Paired-end amplicon sequencing was then carried out on an Illumina MiSeq platform using the v3 reagent kit (Illumina Biotechnology Co, San Diego, USA)

Reads were analyzed using PandaSeq (Masella *et al.*, 2012) to merge forward and reverse reads, quality filtered using USEARCH v8.1.1861 (Edgar, 2010), and taxonomy was assigned using QIIME. Sequences from this study and our previous work doing lab incubated plant litter amended lake sediments (Yakimovich *et al.*, in reveiw) along with representative methanogen sequences from GenBank (Clark *et al.*, 2016) were aligned using MAFFT (Katoh *et al.*, 2002) and alignments were manually edited using MEGA (Kumar *et al.*, 2016). Maximum-likelihood

phylogenetic trees were then constructed with 1000 bootstrap replicates using IQ-TREE (Trifinopoulos *et al.*, 2016). The tree was then colour coded to highlight methanogen orders.

Statistical analysis

Methanogen community (*mcr*A derived) data were analyzed in R v3.3.2 , using the phyloseq package to perform ordination analyses to separate or cluster samples based on differences or similarities in communities (R Core Team, 2016; McMurdie & Holmes, 2013). Indicator species analysis was performed using the Indicspecies package in R via the multi-level pattern analysis (De Caceres and Legendre, 2009) and permutational multivariate analysis of variance using distance matrices (ADONIS) was done using the Vegan package (Oksanen *et al.*, 2017). Linear mixed effect models were constructed to test the effects of our experimental design had on mesocosm chemistry, represnted by pH in our models, and subsequent effects on methanogen relative abundances and methanogenesis. This framework serves as a hypothesis represented in the casual model in our path analysis, and allowed us to identify significant effects in noisy data. Three OTUs were included in the path analysis, OTU 1 and 2 were chosen due to their high abundances in all mesocosms and OTU 9 due to its statistical association with mecosoms containing leaf litter (discusses further in results). The models were constructed using the nlme package in R (Pinheiro *et al.*, 2017) and subsequently used to do a path analysis. Parameter estimates for the path analysis were extracted from the model outputs, and a test for their significance was done with a Kenward-Rogers approximation using the piecwiseSEM package in R (Lefcheck, 2016). Models were assesed using Akaike Information Criterion (AIC) allowing for optimization of our analysis.

Results:

Across all lakes and samples there were 31 distinct methanogen taxa. We observed significant variations in community composition between lakes (ADONIS: $F_{2,251} = 60.713$, $p = 0.001$), and total litter concentrations (ADONIS $F_{2,251} = 20.944$, $p = 0.001$) and day (ADONIS $F_{1,251} = 17.650$, $p = 0.001$), but not between litter qualities (ADONIS $F_{3,251} = 1.288$, $p = 0.259$). Ordination analysis using NMDS showed differences between both the lakes and organic matter concentrations (separation of 95% confidence intervals in Fig. 1), and both of these factors explained more variation in the communities than sampling day according to the ADONIS test (lake $R^2 = 0.28$; concentration $R^2 = 0.096$; day $R^2 = 0.041$). The addition of tree litter regardless of ratio of litter types increased methanogenesis (MANOVA: $F_{3,250} = 28.94$ $p < 0.001$; Tukey's test: $p < 0.001$ for all comparisons of between mesocosms with leaf litter and the controls). Additionally, rates of methanogenesis, were different across lakes, with Swan lake having the highest rates of production, followed by Laurentian and Ramsey (MANOVA: $F_{2,250} = 19.57$ $p < 0.001$; Tukey's test: Laurentian-Ramsey $p = 0.0051$. Laurentian-Swan $p = 0.0069$ and Ramsey-Swan $p < 0.001$).

Temporal trends in sediment chemistry

Mesocosm DOM metrics helped explain differences in decomposition rates between the lakes. The mHIX and FI (Fig. 2) indicated that all mesocosm DOM was primarily terrestrially derived(FI < 1.9; Hansen *et al.*, 2016), and differences in humification between the lakes became more distinct over time (Fig. 2), with less humified DOM on the October sampling date in Swan followed by Ramsey then Laurentian (one-way ANOVA: $F_{2,83} = 164.9$, $p < 0.001$; Tukey's: for all comparisons $p < 0.001$). mHIX was also found to positively correlate with pH ($r = 0.61$, $p <$

0.001) with Swan lake having the lowest pH values followed by Laurentian and Ramsey (one-way ANOVA; $F_{2,256} = 84.18$, $p < 0.001$. Tukey's; for all comparisons with Swan $p < 0.001$, Ramsey – Laurentian $p = 0.02$). Over the three sampling dates the pH generally increased in across all lakes (Fig. 6), whilst CH_4 production increased between August and September across all sites and CO_2 increased in all except for Laurentian (Fig. 3). We also observed much higher light levels reaching the mesocosms in Swan lake compared to Ramsey or Laurentian, ranging from 40-87% depending on the day (summarized by sampling month in Fig. 3). All sites had reduced gas production in October due to the seasonal change and subsequent decrease in temperature (water temperature ranges across all lakes: August 21-26ºC, September 19-23ºC, October 8-11ºC).

Methanogen taxa in the experimental mesocosms

Three taxa were identified as being important in our samples via abundance and indicator species analysis. We found that OTU 1 and 2 dominated all mesocosms, typically making up >90% relative abundances when combined. However, OTU 1 and 2 were strongly and negatively correlated with each other between in all lakes (Fig. 4: Laurentian $r = -0.94$, $p < 0.001$; Ramsey $r = -0.83$ $p < 0.001$; Swan $r = -0.73$ $p < 0.001$), which also correlated with pH (OTU 1 $r = -0.47$, $p < 0.001$; OTU 2 $r = 0.53$ $p < 0.001$). Overall OM loading led to an increase in diversity (measured via chao 1 diversity index) in all lakes (% increase: Laurentian = 44%, Ramsey = 42%, Swan = 49%). This increase in diversity was characterized by newly detectable low abundant methanogens, typically ranging between 0 – 1% relative abundance. However, some of these methanogens showed larger increases in relative abundance, such as OTU 9 (Fig. 4), which

was identified as being associated with litter amendments over all samplings days (Multi-level pattern analysis: $p = 0.001$).

Methanogen diversity

The diversity of methanogen orders in our current study corresponded to the diversity of methanogens in our previous work examining plant litter amendments in lab based incubations (Yakimovich *et al.*, in review). Members from 5 methanogen orders were identified in both experiment with relative even distribution (Fig. 5). In our incubation experiment, OTU 2 was identified as corresponding with high rates of methanogenesis. However in the current field study a closely related methanogen, OTU 292, was not significantly associated with rates of methanogenesis ($r = 0.075$, $p = 0.232$). Phylogenetic analysis revealed that OTU 1 and 2 are both members of the Methanobacteriales and OTU 9 belongs to the Methanomassilococales (Fig. 5). OTU 1's closest related sequence in GenBank is a sequenced clone from an enrichment of rice field soil, and belongs to the family Methanobacteriaceae (Lueders *et al.*, 2001). OTU 2 is closely related to one of two *mcr*A copies found on the genome of *Methanobacterium lacus* AL-21, which was isolated from an acidic fen site in Alaska and was found to be a hydrogenotroph with an optimal growth pH of 6.2 (Cadillo-quiroz *et al.*, 2006). OTU 9 is closely related to *Methanomassiliicoccus luminyensis* B10, which was first isolated from human feces and found to reduce methanol with hydrogen as an electron source to perform methanogenesis (Gorlas *et al.*, 2012).

Path analysis

The path analysis (Fig. 6) revealed a complex network of environmental and biological relationships. Through this analysis, we can see that sampling day, tree litter, and lake had significant effects on pH, while interestingly, concentration of tree litter did not. Additionally, there were only slight differences of the parameter estimates between the litter qualities. Furthermore, we found that OTU 9 had the greatest and only significant effect on methanogenesis out of the OTUs included in the analysis (Fig. 6). However, unlike OTU 1 and 2, pH did not have a significant effect on OTU 9. The path analysis also revealed that OTU 9 had a negative effect on OTU 2, revealing how even a methanogen in low abundance can potentially have an ecological impact and substantially influence C-cycling and CH_4 production in lake sediments.

Discussion:

Our study reveals how terrestrial OM additions can chang methanogen communities and methanogenesis rates. All mesocosms were primarily dominated by Methanobacteriales, irrespective of lake and litter type. We did observe a change in the overall community composition between lakes and after litter additions (Fig. 1), however OTUs 1 and 2 largely dominated the mesocosms. We also saw a lower abundant Methanomassilococales, OTU 9, have a significant effect on methane production in the mesocosms. Higher methanogenesis rates were also associated with faster decomposition rates present in Swan, an overall smaller and less turbid lake the than either Laurentian and Ramsey lake (reflected in light data, Fig. 3). Ratios of deciduous to coniferous tree litters didn't impact community composition or activity over our three study months.

As both OTUs 1 and 2 did not have significant relationships with overall methanogenesis (Fig. 6), we can hypothesize they are low energy scavengers who maintain lower rates of methanogenesis relative to OTU 9. Although OTU 1 and 2 are of the same order, the path analysis shows that they are adapted to different physicochemical conditions. Consistent with being *M. lacus*, OTU 2 was associated with higher pH which was the opposite for OTU 1 (Fig. 6). pH was negatively related with humification of DOM, which shows that the increased rate of decomposition of OM acid filters the methanogen community in favor if OTU 1. In our previous incubation study, we found that a Methanosarcinales, OTU 2, was similarly responsible for higher rates of methanogenesis during decomposition of cattails (*Typha latifolia*) (Yakimovich *et al.*, 2017). However, while present in the mesocosms, no Methanosarcinales OTUs had any significant relationships with methane production. As we observed a wide diversity of methanogens in our study it is likely that genetic plasticity exists in lake sediment methanogen communities, which allows adaptability to differing OM sources.

As decomposition progressed we saw an increase in abundance of OTU 9. Our analysis revealed that pH did not have a direct effect on OTU 9 (Fig. 6). However, as *M. luminyensis* B10 requires methanol to perform methanogenesis, it is likely that OTU 9 has syntrophic bacterial partners that can produce methanol, and are associated directly to pH and decompositional processes. Although first isolated from human feces and associated with gut microbiomes, *M. luminyensis* sequences have been recovered in other environmental samples associated with lower pH systems (e.g. Volant *et al.*, 2012). Our results here show how members from the newest methanogen order, Methanomassilococales, most often associated with gut microbiomes, is an important decomposer in lake sediments.

The different lake conditions also favored different methanogens, as Ramsey and Laurentian lakes mesocosms had higher pHs relative to Swan lake (Fig. 6). The observed lake effect was also reflected in differing decomposition rates, as each lake had distinct humification rates of the added terrestrial organic matter (Fig. 2). In part this was likely due to the differing amounts of light reaching the mesocosms in the different lakes (Fig. 3), with higher light levels measured in Swan corresponding less humified DOM and higher microbial activity (as measured by CO_2 and CH_4 production). With higher amounts of light reaching the mesocosm sediments in Swan the sediments would have increased thermal energy likely accelerating enzymatic activity of microbial decomposers. Additionally, photoexposure of plant derived material has been shown to be able to reduce mHIX values by 8-18% (Hansen *et al.*, 2016), which is another mechanism contributing to the accelerated decomposition in Swan lake.

Conclusion:

The methanogen communities in lake sediments are important as they produce CH_4, a potent greenhouse gas. In this study, we provided novel insights into how terrestrial plant litters influence methanogen community composition and activity. We showed how the decomposition of terrestrial OM selects for different methanogen taxa by changing physicochemical characteristics. Faster decomposition and subsequently greater amounts of CO_2 and CH_4 production were observed in Swan Lake that had less turbid waters and more photoexposed sediment. These results have implications for the role of lakes in global C-cycling, as we observed how different lakes can harbor methanogen communities that differ in composition and activity with equal influxes of terrestrial OM. Understanding methanogen dynamics is integral to

being able to predict lake's role in global c-cycling, directly linking the terrestrial biosphere to the aquatic and subsequently the atmosphere.

References:

Aberg J, Wallin MB. (2014). Evaluating a fast headspace method for measuring DIC and subsequent calculation of pCO(2) in freshwater systems. *Inl WATERS* **4**: 157–166.

Angel R, Claus P, Conrad R. (2011). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. *ISME J* **6**: 847–862.

Antony CP, Colin Murrell J, Shouche YS. (2012). Molecular diversity of methanogens and identification of Methanolobus sp. as active methylotrophic Archaea in Lonar crater lake sediments. *FEMS Microbiol Ecol* **81**: 43–51.

Biderre-Petit C, Jézéquel D, Dugat-Bony E, Lopes F, Kuever J, Borrel G, *et al.* (2011). Identification of microbial communities involved in the methane cycle of a freshwater meromictic lake. *FEMS Microbiol Ecol* **77**: 533–545.

Billard E, Domaizon I, Tissot N, Arnaud F, Lyautey E. (2015). Multi-scale phylogenetic heterogeneity of archaea, bacteria, methanogens and methanotrophs in lake sediments. *Hydrobiologia* **751**: 159–173.

Borrel G, Lehours AC, Crouzet O, J??z??quel D, Rockne K, Kulczak A, *et al.* (2012). Stratification of Archaea in the deep sediments of a freshwater meromictic lake: Vertical shift from methanogenic to uncultured Archaeal lineages. *PLoS One* **7**. e-pub ahead of print, doi: 10.1371/journal.pone.0043346.

De Caceres M, Legendre P. (2009). Associations between species and groups of sites: indices and statistical inference. *Ecology* **90**: 3566–3574.

Cadillo-quiroz H, Yashiro E, Yavitt JB, Zinder SH, Bra SL. (2006). Isolation of a novel acidiphilic methanogen from an acidic peat bog. *Nat Lett* **442**: 192–195.

Chim Chan O, Claus P, Casper P, Ulrich A, Lueders T, Conrad R. (2005). Vertical distribution

of structure and function of the methanogenic archaeal community in Lake Dagow sediment. *Environ Microbiol* **7**: 1139–1149.

Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. (2016). GenBank. *Nucleic Acids Res* **44**: D67–D72.

Danielsson R, Dicksved J, Sun L, Gonda H, Müller B, Schnürer A, *et al.* (2017). Methane Production in Dairy Cows Correlates with Rumen Methanogenic and Bacterial Community Structure. *Front Microbiol* **8**: 1–15.

Edgar RC. (2010). Search and clustering orders of magnitude faster than BLAST. *BIOINFORMATICS* **26**: 2460–2461.

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey DW, *et al.* (2007). Changes in Atmospheric Constituents and in Radiative Forcing. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, et al. (eds). *Climate Change*. Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA. https://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter2.pdf.

Glissmann K, Chin KJ, Casper P, Conrad R. (2004). Methanogenic pathway and archaeal community structure in the sediment of eutrophic Lake Dagow: Effect of temperature. *Microb Ecol* **48**: 389–399.

Gorlas A, Robert C, Gimenez G, Drancourt M, Raoult D. (2012). Complete genome sequence of Methanomassiliicoccus luminyensis, the largest genome of a human-associated Archaea species. *J Bacteriol* **194**: 4745.

Hansen AM, Kraus TEC, Pellerin BA, Fleck JA, Downing BD, Bergamaschi BA. (2016).

Optical properties of dissolved organic matter (DOM): Effects of biological and photolytic degradation. *Limnol Oceanogr* **61**: 1015–1032.

Hofmann H, Federwisch L, Peeters F. (2010). Wave-induced release of methane : Littoral zones as a source of methane in lakes. *Limnol Oceanogr* **55**: 1990–2000.

Katoh K, Misawa K, Kuma K, Miyata T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* **30**: 3059–3066.

Kritzberg ES, Cole JJ, Pace ML, Granéli W, Darren L, Kritzberg ES, *et al.* (2004). Autochthonous versus allochthonous carbon sources of bacteria : Results from whole-lake 13C addition experiments. *Limnol Oceanogr* **49**: 588–596.

Kumar S, Stecher G, Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* **33**: 1870–1874.

Lefcheck JS. (2016). piecewiseSEM: Piecewise structural equation modeling in R for ecology, evolution, and systematics. *Methods Ecol Evol* **7**: 573–579.

Lueders T, Chin KJ, Conrad R, Friedrich M. (2001). Molecular analyses of methyl-coenzyme M reductase α-subunit (mcrA) genes in rice field soil and enrichment cultures reveal the methanogenic phenotype of a novel archaeal lineage. *Environ Microbiol* **3**: 194–204.

Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. (2012). PANDAseq : PAired-eND Assembler for Illumina sequences. *BMC Bioinformatics* **13**: 1–7.

McMurdie PJ, Holmes S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One* **8**.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, *et al.* (2017). vegan: Community Ecology Package. https://cran.r-project.org/package=vegan.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. (2017). nlme: Linear and Nonlinear

Mixed Effects Models. https://cran.r-project.org/package=nlme.

R Core Team. (2016). R: A language and environment for statistical computing. *R Found Stat Comput*. https://www.r-project.org/.

Schwarz JIK, Eckert W, Conrad R. (2007). Community structure of Archaea and Bacteria in a profundal lake sediment Lake Kinneret (Israel). *Syst Appl Microbiol* **30**: 239–254.

Stoeva MK, Aris-Brosou S, Chételat J, Hintelmann H, Pelletier P, Poulain AJ. (2014). Microbial community structure in lake and wetland sediments from a high arctic polar desert revealed by targeted transcriptomics. *PLoS One* **9**: 1–13.

Tanentzap AJ, Szkokan-Emilson EJ, Desjardins CM, Orland C, Yakimovich KM, Dirszowsky R, *et al.* (2017). Bridging between litterbags and whole-ecosystem experiments: a new approach for studying lake sediments. *J Limnol*. e-pub ahead of print, doi: 10.4081/jlimnol.2017.1588.

Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ. (2016). W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res* **44**: 232–235.

Volant A, Desoeuvre A, Casiot C, Lauga B, Delpoux S, Morin G, *et al.* (2012). Archaeal diversity: temporal variation in the arsenic-rich creek sediments of Carnoules Mine, France. *Extremophiles* **16**: 645–657.

West WE, Coloso JJ, Jones SE. (2012). Effects of algal and terrestrial carbon on methane production rates and methanogen community structure in a temperate lake sediment. *Freshw Biol* **57**: 949–955.

Yakimovich KM, Szkokan-Emilson EJ, Carson MA, Tanentzap AJ, Basiliko N, Mykytczuk NCS. (2017). Plant litter type dictates microbial communities responsible for greenhouse gas production in lake sediments. *ISME J* **In review**.

Yang Y, Li N, Wang W, Li B, Xie S, Liu Y. (2017). Vertical profiles of sediment methanogenic potential and communities in two plateau freshwater lakes. *Biogeosciences* **14**: 341–351.

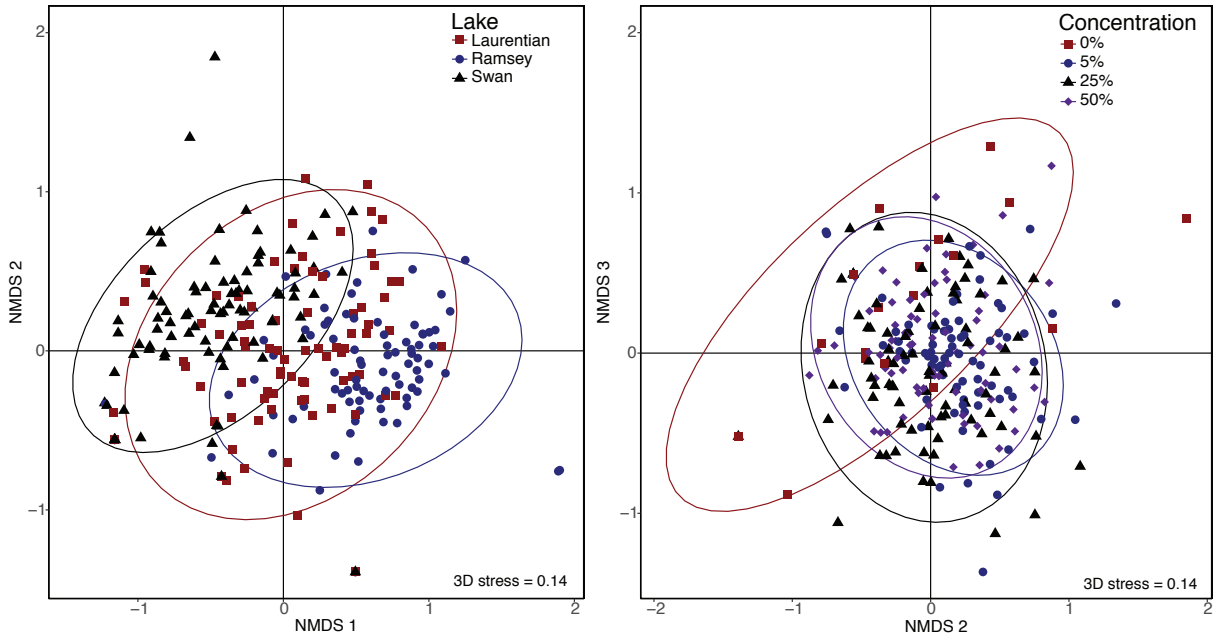


Figure 3.1: Mesocosm methanogen communities differ across lakes and OM concentrations. Plots are axes from a NMDS of methanogen community composition using Bray-Curtis distance matrices. The left panel is displaying axis 1 and 2 with samples grouped by lake and the right panel is displaying axis 2 and 3 with samples grouped my concentration (% OM) of added tree litters. Ellipses represent 95% confidence intervals, and stress values are in the lower right hand corner of the plots.

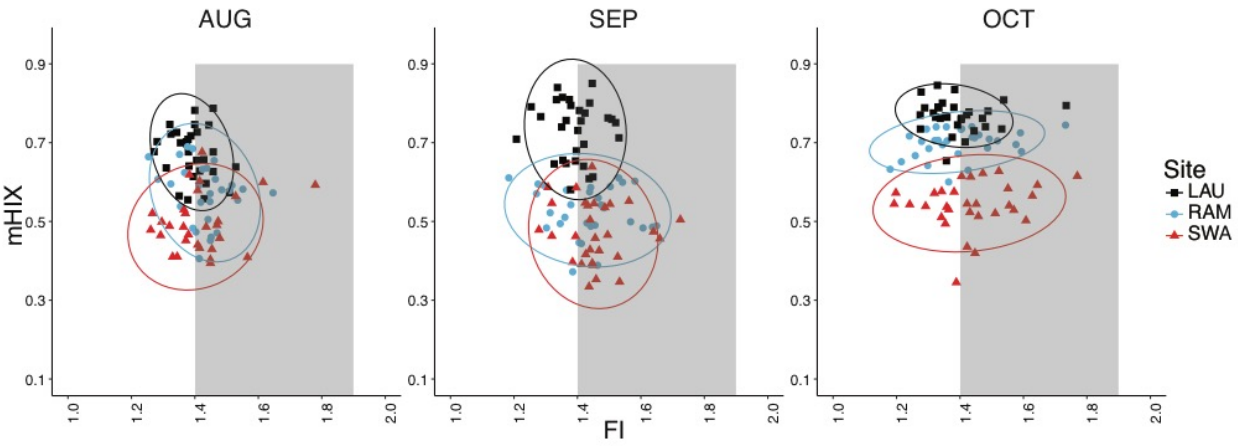


Figure 3.2: mHIX (humification index) versus FI (fluorescence index) plots of all lakes across each sampling date. Each point represents a single mesocosm from the respective sampling date and lake. Ellipses represent 95% confidence intervals. FI values to the left of the grey bar represent samples with a terrestrial derived C signal and to the right indicates a microbial derived C signal.

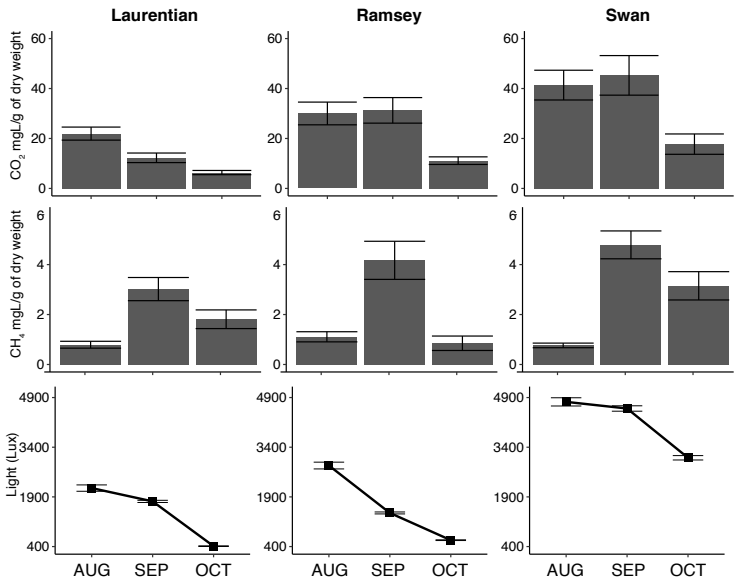


Figure 3.3: Temporal trends in pore water. Mean ± standard error for (A) pore water CO_2, (B) pore water CH_4, and (C) light level reaching the top of the mesocosm at each sampling date in each lake.

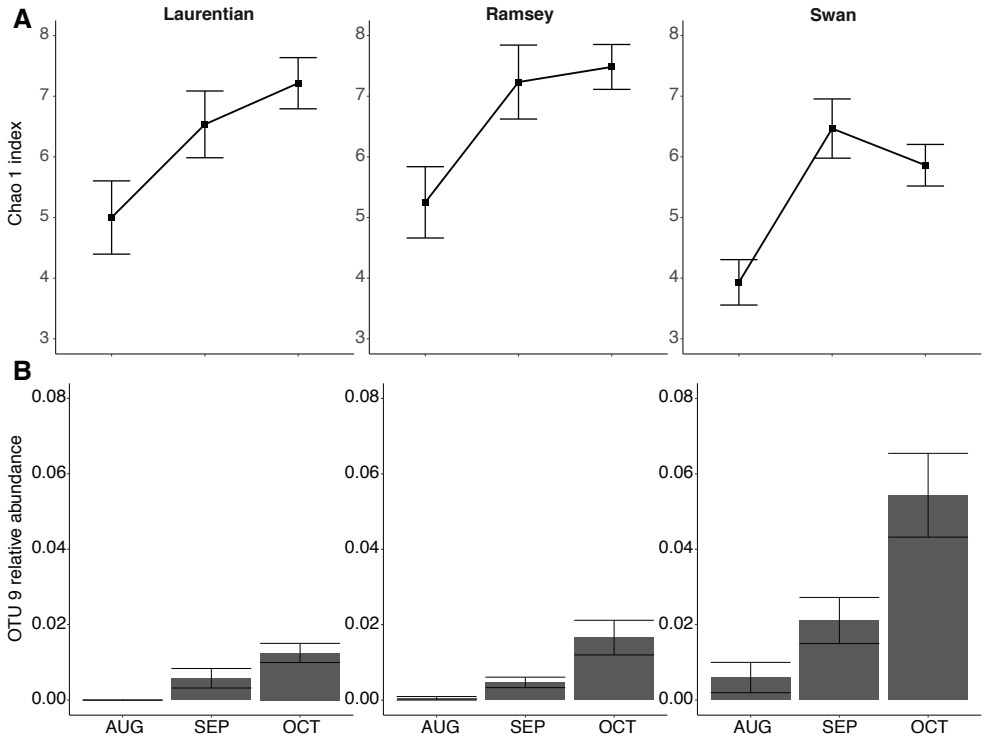


Figure 3.4: Chao 1 diversity and abundance of OTU 9 increase over time across the experiment. Mean ± standard error for (A) Chao 1 diversity index and (B) relative abundance of OTU 9 over sampling days in each lake.

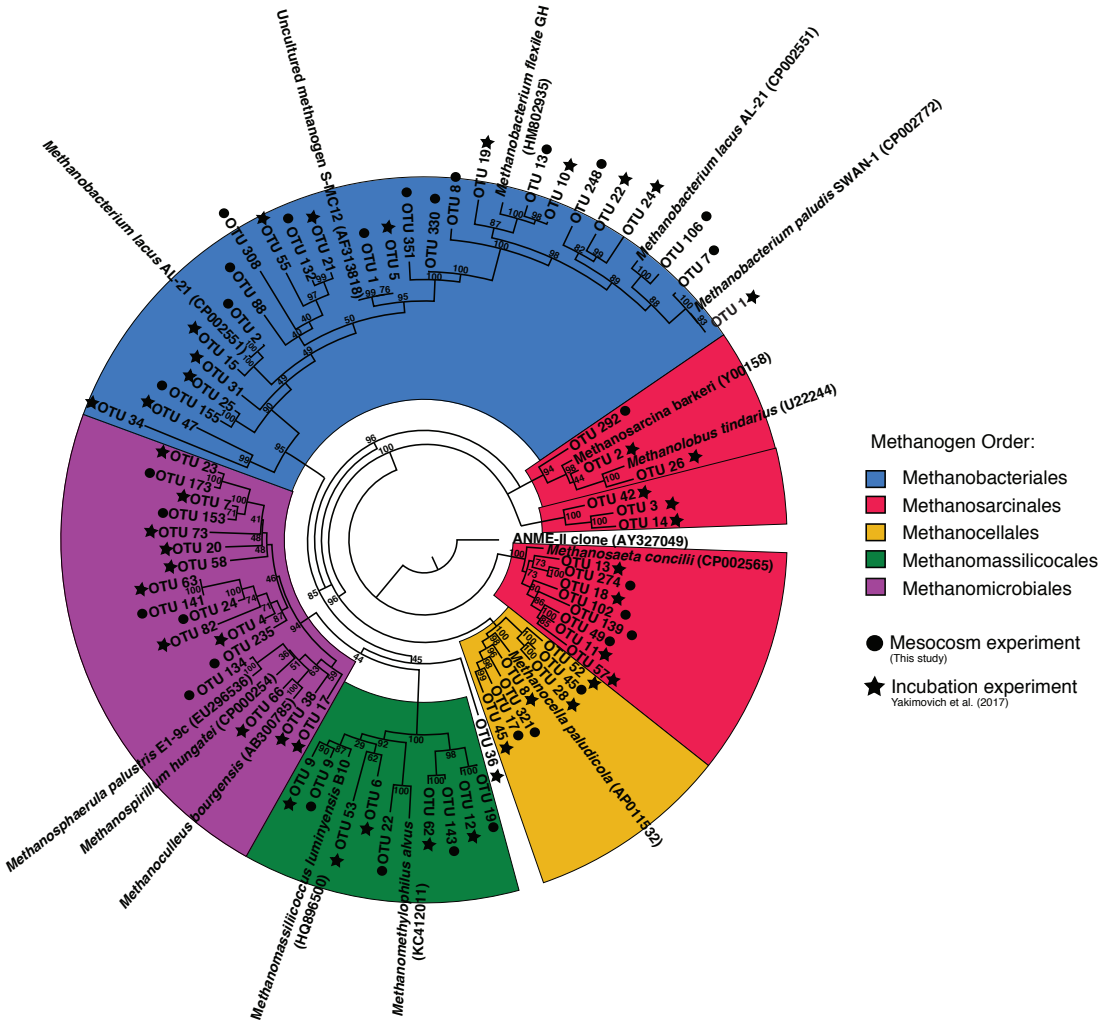


Figure 3.5: A phylogenetic tree of all OTUs from the mesocosms. This tree was constructed using maximum likelihood with 1000 bootstraps, which are displayed on the nodes. Taxa were grouped and colour coded by methanogen order and relevant genome sequenced methanogen species were included using data from GenBank. Sequences were also added from a previous lab incubation study (Yakimovich et al. 2017) to allow comparison of methanogen diversity.

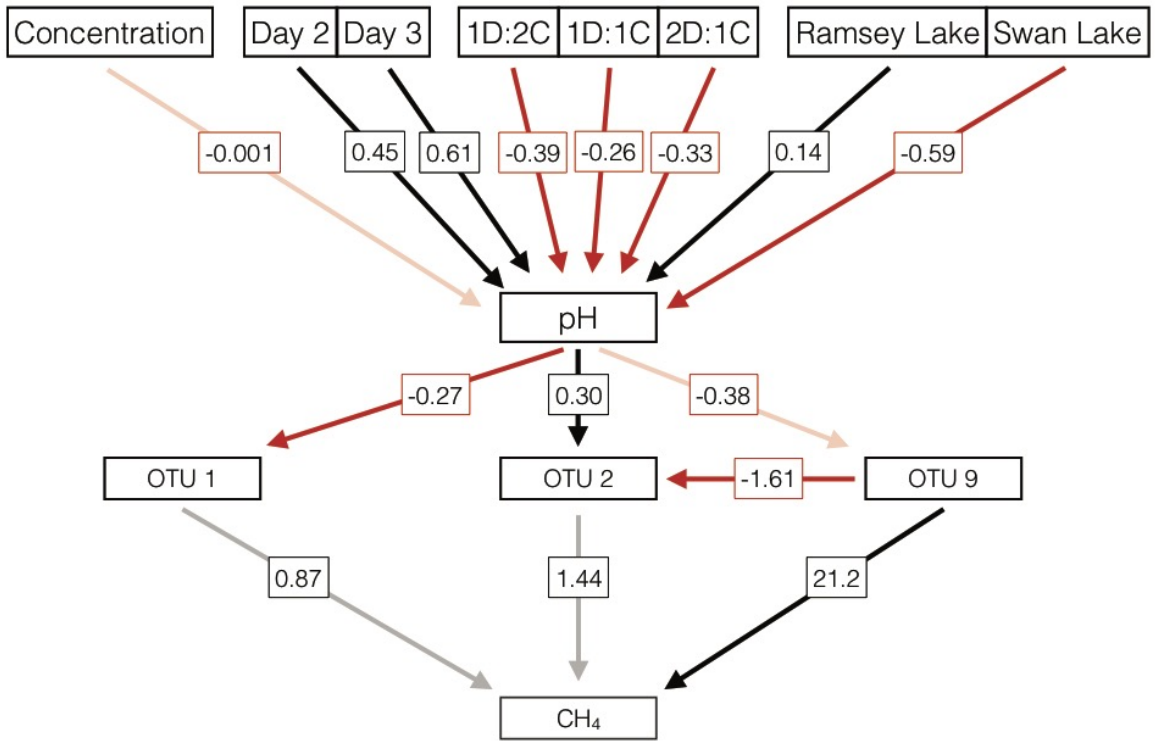


Figure 3.6: Path diagram displaying effects of the mesocosm physiochemical and microbial parameters on CH_4 production. Black arrows represent positive effects and red represent negative effects, with parameter estimates in the boxes. Arrows that are faded represent non-significant effects ($p > 0.05$). Litter qualities are represented as a ratio of D (deciduous): C (coniferous) litter. As the models are relative all discrete variables use a factor level as an intercept (zero point) and therefore do have an effect parameter, for Day it is Day 1, for litter quality it is the controls, for lakes it is Lake Laurentian.

General conclusions:

In this thesis, I explored the microbial decomposer communities in lake sediments, with a special interest in methanogens and their activity. In an ecological context, methanogens are terminal organic matter (OM) decomposers and work in tight symbiosis with other microbes. In chapter 1 I reviewed the body of literature exploring the diversity and functioning of sediment microbial communities. It is evident that lakes provide different microbial habitats based on both differences in available substrates/metabolites and in other physicochemical characteristics. The review also reveals that presently there is a sparse understanding of how specific OM sources influence sediment microbial community composition and function.

In chapter 2 I used lake sediments that were amended with different plant litters *in-vitro* to study microbial community responses. By using different OM types, it was observed that polyphenolic compounds in the pore water that were released from the plant litters drove differences in decomposer communities. A second set of identical incubations was also prepared with the addition of a 5% spike of higher OM content sediments, which revealed a legacy effect from different microbial communities present in the spiked versus un-spiked sediments. Differences in polyphenol concentrations selected for different dominant bacterial and methanogen orders, and I saw increases in overall abundance of fungi. Additionally, the spiked sediments that contained the highest concentration of polyphenols also had different dominant bacterial taxa than either the lower concentration spiked or un-spiked sediments. While fungal diversity largely was dictated by litter type, polyphenol concentration correlated with increases in white-rot fungal taxa, which are known to produce polyphenol oxidases, but not typically found in strictly anoxic environments. The changes seen across bacterial, fungal and methanogen

communities in response to plant litter types helps illustrate just how adaptable microbial communities may be to changing plant communities in lake catchments.

Understanding how terrestrial OM inputs affect sediment microbial communities is important, as terrestrially derived C is typically slower to decompose than autochthonous C and thus contributes to C storage potential in lake sediments. In chapter 1 I saw how, in a closed system, the buildup of polyphenols had a large impact on microbial communities, including the inhibition of methane production, despite otherwise overall lability of OM shown via CO_2 production. In chapter 3 I collected and analyzed data from a field experiment with artificially constructed sediment mesocosm across 3 lakes. This *in-situ* setup exposed the mesocosms to differing physical, chemical, and ecological factors. Notably, the mesocosms experienced natural solar radiation, in contrast to the lab incubations, and were open to fluxes of water and dissolved chemicals/nutrients across the different lakes. The mesocosms in the smallest and least turbid of the study lakes, Swan Lake, had more photoexposure than either of the other lakes. Subsequently, I observed less humified DOM in the sediment pore waters and more CO_2 and CH_4 production. Litter decomposition in the mesocosms also lowered the pH, which selected for different methanogen taxa. Additionally, as decomposition progressed I saw increases of a Methanomassilococales spp. taxon that was responsible for increased rates of methanogenesis.

By reviewing existing literature, and from the results of both experiments conducted for my MSc project, I conclude that lake sediment microbial communities change their composition and activities in response to varying sources of OM. The results here also provide valuable insights via constrained experiments that can form important future hypotheses for future landscape-scale studies examining how terrestrial plant communities in catchments and/or coastal areas alter sediment biodiversity and functioning; for example, in the context of

watershed management during land use changes such as urbanization or agricultural and industrial development. The studies presented in this thesis also demonstrate that with changing OM sources, sediment microbial communities can shift, and there are consequences for rates of OM mineralization. This underscores the importance of research in freshwater lake sediments, as they serve as "bioreactors" for incoming C-sources from the surrounding landscape, which is subject to changes from a variety of stressors. Data presented here also help present a picture of lake sediments as microbial habitats, with a wide diversity of bacteria, fungi and methanogens. Together these microbial communities work in complex symbioses to decompose OM influxes to lake sediments, and dictate the fate of that C.